Setting-up of a 24 h model to evaluate the activity of antibiotics against intracellular forms of S. aureus infection


Unité de Pharmacologie cellulaire et moléculaire
Université catholique de Louvain
Brussels, Belgium
Why intracellular S. aureus?

- facultative intracellular organism
  - macrophages, monocytes, PMN
  - endothelial cells…
- remains hidden for prolonged periods
- is supposedly protected against many antibiotics
  - exposed to concentrations that do not allow fast killing…
  - is in a metabolic situation that make less susceptible to antibiotics
- may be a cause of recurrence / resistance!!
Setting-up of the model: J774 mouse macrophages

- infection of macrophages (0.5 bact/cell)
- washing with gentamicin 50 µg/ml
- incubation for up to 24 h with
  - gentamicin 0.5 µg/ml (MIC) for controls
  - antibiotic under study alone for tests
- the AB must be able to cope with both extracellular and intracellular S. aureus!! at clinically-meaningful concentrations (MIC to C\text{max})

Data from Seral et al. AAC 2003 47:2283-2292
Validation of the model: confocal microscopy

5 hours infection

no antibiotic  gentamicin 0.5 mg/L

data from Seral et al. AAC 2003 47:2283-2292
Validation of the model: electron microscopy
Validation of the model: S. aureus growth in controls
Use of the model to test antibiotic intracellular activity

adapted from Seral et al. AAC 2003 47:2283-2292
An improved model: THP-1 human macrophages

- behavior ~ as human monocytes
- valuable model for
  - AB activity testing
  - cytokine effect testing

Auwerx, Experientia, 1990 47:22-31

- infection of macrophages (4 bact/cell)
- washing with GEN 50 µg/ml
- incubation for up to 24 h with
  - GEN 0.5 µg/ml (MIC)
  - antibiotic under study
Aminoglycoside activity: concentration-effect relation for gentamicin

- Highly bactericidal
- Concentration dependent

- Poorly bactericidal
- Concentration independent
Aminoglycoside activity: possible reasons for loss of activity

PHARMACOKINETIC PARAMETERS
2. Accumulation
   too slow? Probably NO!
   (preloading ineffective ...)

PHARMACODYNAMIC PARAMETERS
5. Expression of activity
   acidity? YES!
   MIC pH 5 = 32 X MIC pH 7
Beta-lactam activity: concentration-effect relation for oxacillin

- More slowly bactericidal
  - concentr. independent

- slowly acting
  - concentr. dependent
Beta-lactam activity: concentration-effect relation for oxacillin and relation to clinical dosings

Intracellular (?) β-lactams are concentration-dependent down to 2 log killing!!

400 X MIC!
Beta-lactam activity at Cmax

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (ug/ml)</th>
<th>Cmax (ug/ml)</th>
<th>Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contr</td>
<td>0.125</td>
<td>63</td>
<td>&lt;1</td>
</tr>
<tr>
<td>OXA</td>
<td>0.06</td>
<td>48</td>
<td>&lt;1</td>
</tr>
<tr>
<td>AMP</td>
<td>0.015</td>
<td>6.3</td>
<td>&lt;1</td>
</tr>
<tr>
<td>PEN V</td>
<td>0.25</td>
<td>40</td>
<td>&lt;1</td>
</tr>
<tr>
<td>NAF</td>
<td></td>
<td></td>
<td>&lt;1</td>
</tr>
</tbody>
</table>
Oxacillin activity: possible reasons for (loss of) activity

### PHARMACOKINETIC PARAMETERS

- **2** Accumulation could be compensated by higher $C_e$ ...
- **3** Subcellular distribution partial redistribution?

### PHARMACODYNAMIC PARAMETERS

- **6** Bacterial responsiveness slowly growing bacteria ...
- **7** Cooperation with host defenses was proposed to explain activity*

* Van den Broek et al. JAC 1986 17: 767-774
Macrolide activity: concentration-effect relation for telithromycin

- Limit. bactericidal effect
- Concentr. independent

- Static only
- Concentr. independent
Macrolide activity at Cmax

![Bar chart showing the comparison of log CFU values for Contr, AZM, and TELITHRO in both intra and extra conditions.]

**Table:**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>MIC (ug/ml)</th>
<th>Cmax (ug/ml)</th>
<th>Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contr</td>
<td>0.5</td>
<td>0.5</td>
<td>38</td>
</tr>
<tr>
<td>AZM</td>
<td>0.06</td>
<td>2</td>
<td>28</td>
</tr>
<tr>
<td>TELITHRO</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Macrolide activity:
possible reasons for loss of activity

- Expression of activity
  acidity? YES!
  MIC pH 5 = 100 X MIC pH 7
  bacteriostatic

PHARMACOKINETIC PARAMETERS

PHARMACODYNAMIC PARAMETERS

$C_c > 30 \times C_e$
Quinolone activity: concentration-effect relation for moxifloxacin

- Bactericidal
- Concentration-dependent
  - But more slowly acting
  - And limited to 2-3 log
Quinolone activity at Cmax

The graph shows the change in log CFU from t0 to t, indicating the effect of different antibiotics on bacterial growth at their peak concentration (Cmax).

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC (µg/ml)</th>
<th>Cmax (µg/ml)</th>
<th>Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contr</td>
<td>0.06</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>MOXI</td>
<td>&lt;0.03</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>GARENO</td>
<td>0.125</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>LEVO</td>
<td>0.125</td>
<td>4.3</td>
<td>5</td>
</tr>
<tr>
<td>CIPRO</td>
<td>0.125</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

The table provides the minimum inhibitory concentration (MIC) and peak concentration (Cmax) values for each antibiotic along with the accumulation at Cmax.
Quinolone activity: possible reasons for reduced activity

\[ Cc > 5 \times Ce \]

PHARMACOKINETIC PARAMETERS

3. Subcellular distribution
   - Soluble fraction?
   - Highly diffusible and relocatable

4. Inactivation
   - Binding to cell constituents?

PHARMACODYNAMIC PARAMETERS

5. Expression of activity
   - Acidity? YES!
   - MIC pH 5 = 4-8 X MIC pH 7
Oritavancin: concentration-effect relationships

- Highly bactericidal
- Concentration-dependent
- More slowly acting
- But still bactericidal
- Concentration-dependent
Comparison vancomycin - oritavancin

vancomycin: Slowly cidal extracellularly; mostly static intracellularly.

oritavancin: Rapidly cidal extracellularly; More slowly but still cidal intracellularly at 24h.
Glycopeptide activity at Cmax

<table>
<thead>
<tr>
<th></th>
<th>Contr</th>
<th>TEICO</th>
<th>VANCO</th>
<th>ORITA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ log CFU (t-t0)</td>
<td>0.00</td>
<td>1.00</td>
<td>0.50</td>
<td>0.25</td>
</tr>
<tr>
<td>MIC (ug/ml)</td>
<td>0.25</td>
<td>1.00</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Cmax (ug/ml)</td>
<td>100</td>
<td>50</td>
<td>25</td>
<td>148</td>
</tr>
<tr>
<td>Accumulation</td>
<td>7</td>
<td>6</td>
<td>148</td>
<td>148</td>
</tr>
</tbody>
</table>
Oritavancin activity: possible reasons for loss of activity

Cc > 150 X Ce

PHARMACOKINETIC PARAMETERS

4. Inactivation
   Binding to cell constituents ?

PHARMACODYNAMIC PARAMETERS

5. Expression of activity
   acidity ? NO !
   MIC pH 5 = MIC pH 7
Antibiotic activity: summary

NCCLS definition of bactericidal activity
And we know since 1993 that Synercid accumulates in cells ...

adapted from Carryn et al 2003, Infect Dis Clin N Am
And here is the team….

Cristina Seral

Maritza Barcia-Macay

Françoise Van Bambeke

Marie-Paule Mingeot-Leclercq

Paul M. Tulkens

visit www.antiinfectieux.org

And visit also the ISAP web site: www.isap.org