Magic activity of β-lactams against intracellular Methicillin-Resistant S. aureus: role of acidic pH

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Methicillin Resistant S. aureus (MRSA)

- Reduced susceptibility to β-lactams
- Acquisition of a low-affinity Penicillin Binding Protein 2a (transpeptidase activity)
- Exposition to a low pH (5.0 to 5.5) has been shown to significantly reduce methicillin resistance

(Sabath et al, AAC, 1972; Lemaire et al, AAC, 2007)
S. aureus sojourns in various acidic environments

- S. aureus can grow and divide normally in a broad pH range (5 to 9)

- S. aureus survives in various acidic environments, such as the mouth, the skin, the urinary tract, the vagina, and various types of eukaryotic cells

Weinrick et al, J. Bacteriol., 2004
Part I. Role for intracellular infections
Intracellular *S. aureus*

- **Complicated Skin Infections**

- **Pulmonary Infections Associated with Cystic Fibrosis**

- **Endocarditis**
  - Sinha and Herrmann, *Thromb Haemost.*, 2005

- **Osteomyelitis**

- **Recurrent Rhinosinusitis**
Intracellular lifestyle of *S. aureus*

- **FnBPs**
- **Fibronectin**
- **Integrins**
- **Cells**
- **Phagosomes**
- **Lysosomes**
- **Phagolysosomes**

**Cytosol (~ pH 7.0)**

**Endothelial cells**
**Osteoblasts**

**pH 4.5 to 5.5**
Restored activity of cloxacillin against intr. MRSA

In phagolysosomal model of infection, cloxacillin shows similar activity against both MSSA and MRSA

* MSSA, ATCC 25923 ; MRSA, ATCC 33591

Lemaire et al, AAC, 2008
Restored activity of cloxacillin against intr. MRSA

Restoration of β-lactam activity can be extended to MRSA isolates of current clinical and epidemiological interest

Lemaire et al, AAC, 2008
Role of acidic pH (i)

- Ammonium chloride, a lysosomotropic agent, neutralizes the pH of lysosomal organelles

- Neutralization of the lysosomal pH makes MRSA insensitive to the action of both cloxacillin and meropenem
In endothelial cells (HUVEC cell line)

- *S. aureus* gains access to the cytosol (where pH is not in the acid)
- Cloxacillin shows less activity towards MRSA compared to MSSA
Part II. Role for the bacteria
A. Binding to PBPs

- The influence of pH on total penicillin-binding properties was measured with intact bacteria using BOCILLIN FL, a fluorescent derivative of penicillin V.

- MRSA grown in acidic conditions shows a larger binding of BOCILLIN FL compared to bacteria grown in neutral conditions.

- No significant difference is noted between MSSA and MRSA for bacteria grown at pH 5.5.
B. Studies with PBP2a (i)

INFLUENCE OF PH ON THE BINDING TO PBP2a

At a lower pH, PBP2a binds β-lactams more avidly and more rapidly

Lemaire et al, JBC, 2008
B. Studies with PBP2a (iii)

CIRCULAR DICHROISM SPECTRA

At pH 5.5: PBP2a undergoes a conformational change in the presence of oxacillin, consistent with the opening of the active site.

Lemaire et al, JBC, 2008
Conclusions: PBP2a

**pH 7.0**

PBP2a: closed conformation of the active site

- Poor access by the antibiotic to the active site of the protein
- Poor inhibition of PBP2a by β-lactam, conferring resistance to these agents

**pH 5.5**

PBP2a: open conformation of the active site

- Improved access by the antibiotic to the active site of the protein
- Increased inhibition of PBP2a by β-lactam
Acknowledgments

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B. Studies with PBP2a (ii)

INFLUENCE OF PH ON KINETIC PARAMETERS OF INTERACTIONS BETWEEN NITROCEFIN AND PBP2A

\[
\begin{align*}
E + N & \rightleftharpoons EN \\
\text{Kd} & \quad k_2 \\
EN & \rightarrow E - N \\
k_3 & \\
E - N & \rightarrow E-P
\end{align*}
\]

<table>
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<tr>
<th>Parameters</th>
<th>pH</th>
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<tbody>
<tr>
<td></td>
<td>7.0</td>
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<tr>
<td>( K_d ) (( \mu \text{M} ))</td>
<td>195 ± 28</td>
</tr>
<tr>
<td>( k_2 ) (s(^{-1}) ( \times 10^3 ))</td>
<td>6.0 ± 0.6</td>
</tr>
<tr>
<td>( k_3 ) (s(^{-1}) ( \times 10^6 ))</td>
<td>2.5 ± 0.2</td>
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Lemaire et al, JBC, 2008
B. Studies with PBP2a (iv)

CIRCULAR DICHROISM SPECTRA

At pH 5.5 : PBP2a undergoes a conformational change in the presence of oxacillin, consistent with the opening of the active site

Lemaire et al, JBC, 2008