Comparison of the intracellular and extracellular activities of approved and novel antistaphylococcal antibiotics using a pharmacodynamic model exploring full drug concentration-responses.

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1. Background and Model

Infections caused by Staphylococcus aureus remain a therapeutic challenge, due, in part, to the ability of these organisms to survive in intracellular compartments of a variety of eukaryotic cells.

In this context, our laboratory has undertaken to systematically measure and compare the activities of a large array of antibiotics (approved and in development) from different pharmacological classes against the intracellular and extracellular forms of S. aureus, using a pharmacodynamic model allowing for a quantitative assessment of their concentration-dependent effects in these environments.

In brief, S. aureus isolates (susceptible to the antibiotics studied) are either (i) placed in broth (extracellular bacteria), or (ii) phagocytized by THP-1 monocytes (intracellular bacteria; non-phagocytized bacteria are removed by washing and short-term exposure of cells to gentamycin (100 x MIC)). Antibiotics are then added to the broth or to the monocytes culture medium for 24 h at concentrations ranging typically from 0.1 to 100 x the MIC to obtain full concentration-dependent responses and to calculate two pertinent pharmacodynamic parameters based on Hill's equation ([sigmoidal function]).

The figure shows a typical example of such a response for extracellular and intracellular forms of different S. aureus strains to an experimental antibiotic (peptide deformylase) and the parameters analyzed using different strains (compared at equipotent concentrations).

2. Observations (equipotent concentrations [MIC normalized graphs])

1. data with ATCC 25923

- Extracellular bacteria (broth)
- Intracellular bacteria

2. data with multiple strains and ceftaroline (as an example)

- Extracellular bacteria (broth)
- Intracellular bacteria

3. Pharmacodynamic parameters (from Hill's equation)

<table>
<thead>
<tr>
<th>antibiotic</th>
<th>strain</th>
<th>Emax (A log10 CFU)</th>
<th>Cmax (multiple of MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ceftaroline</td>
<td>ATCC33591</td>
<td>-5.3</td>
<td>0.5</td>
</tr>
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<td>multiple strains</td>
<td>-5.1</td>
<td>0.7</td>
</tr>
<tr>
<td>daptomycin</td>
<td>ATCC33591</td>
<td>-5.1</td>
<td>0.15</td>
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<td>-5.1</td>
<td>0.15</td>
</tr>
<tr>
<td>gepotidacin</td>
<td>ATCC25923</td>
<td>-5.1</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>multiple strains</td>
<td>-5.1</td>
<td>1.3</td>
</tr>
<tr>
<td>moxifloxacin</td>
<td>ATCC25923</td>
<td>-4.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

4. Discussion and Conclusions

- All antibiotics tested, except moxifloxacin, show a sharp decrease of their intracellular maximal relative efficacy (Emax) compared to broth, and are not bactericidal in cells (less than 3 log10 CFU decrease compared to the post-phagocytosis inoculum) even though all are bactericidal in broth (3 log10 CFU or more);

- As Emax is the value extrapolated for an infinitely large extracellular concentration of antibiotics, this loss of efficacy cannot be due to a simple global lack of diffusion and accumulation of the antibiotics.

- Conversely, all antibiotics tested show similar relative potencies (Cmax), in the range of their MIC in broth. This shows that intracellular bacteria are as susceptible as those in broth, indicating that the antibiotics tested are able to penetrate cells and reach their target (at least in part).

- We showed (not illustrated here) that intracellular S. aureus that remained in cells (i) are not Small Colony Variants (except in rare cases), and (ii) show an unaltered MIC when retested in broth.

- Intracellular bacteria, for a low but significant proportion of the original inoculum, are probably in a state of non-responsiveness to antibiotics.