Activity of the novel Fabl inhibitor Debio 1452 against intracellular forms of susceptible and resistant *S. aureus*: comparison with linezolid, vancomycin and daptomycin

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Background and Aims

Staphylococcus aureus remains a therapeutic challenge, due in part to the ability of this organism to acquire resistance mechanisms to most recommended antibiotics (1) and to survive in intracellular compartments of eukaryotic cells (2). In this context, it is therefore essential (i) to foster the discovery and development of novel antibiotics with mode(s) of action distinct from those in current use, and (ii) to assess the activity of these molecules against intracellular *S. aureus*.

Debio 1452 (formerly AFN 1252; see structure in Figure 1) is the active agent of Debio 1450 (2). In this context, it is therefore essential (i) to foster the discovery and development of novel antibiotics with mode(s) of action distinct from those in current use, and (ii) to assess the activity of these molecules against intracellular *S. aureus*. Debio 1452 (formerly AFN 1252; see structure in Figure 1) is the active agent of Debio 1450 (2).

The goal of our study was to compare the intrinsic activity (MIC) and the intracellular activity of the active molecule of Debio 1452 (with that of other anti-staphylococcal agents against a series of strains with different drug phenotypes.

**Figure 1**: structure and main biochemical properties of Debio 1452

Methods

**Bacterial strains and MIC measurements**

- *S. aureus* reference strain ATCC 29523 (MSSA) and resistant strains NRS119, MU50 and SA404.
- LZD were obtained as indicated in the Table and grown in MBH as previously described (4).
- MICs were determined according to CLSI recommendations (4) and interpreted using available EUCAST clinical breakpoints (5).

**Intracellular activity**

- Experiments were performed with human THP-1 monocytes, displaying monophagocytic-like activity (6).
- Phagocytosis of opsonized bacteria was allowed for 1 h using a 4:1 bacteria-macrophage ratio, followed by elimination of extracellular bacteria by 45 min exposure to gentamicin (50 mg/L) and addition of the antibiotic to extracellular concentrations varying from at least 1/100 to 100x the MIC to the MHB to obtain full dose-response.
- Intracellular activity is expressed as the change in the initial inoculum as extrapolated for an infinitely large inoculum as follows:

\[
\log_{10}\text{CFU (30 h)} = \log_{10}\text{CFU (0 h)} + \log_{10}\text{ICU (inoculum as extrapolated for an infinitely large inoculum)}
\]

Where ICU (inoculum as extrapolated for an infinitely large inoculum) is calculated using the following formula:

\[
\text{ICU} = \frac{(10^X - 1)}{(10^X - 10^5)} 
\]

where X is the log MIC in broth, which suggests a free penetration and an effective access to its bacterial target in phagocytes.

**Pharmacodynamic model used in this study: analysis of the data**

Data are used to fit a Hill equation allowing to determine the two key pharmacological descriptors of antibiotic activity (C<sub>50</sub> and E<sub>max</sub>) as described in Figure 2.

**Figure 2**: Pharmacodynamic model used in this study: analysis of the data

- Data are used to fit a Hill equation allowing to determine the two key pharmacological descriptors of antibiotic activity (C<sub>50</sub> and E<sub>max</sub>) as described in Figure 2.

**Discussion and Conclusions**

- Debio 1452 is active against *S. aureus* phagocytized and thriving in human THP-1 monocytes, disregarding their resistant phenotypes to other currently used anti-staphylococcal antibiotics.
- The intracellular relative potency (C<sub>50</sub>) of Debio 1452 is close to its MIC in broth, which suggests a free penetration and an effective access to its bacterial target in phagocytes.
- The data suggest that Debio 1452 may constitute a useful alternative to most anti-staphylococcal agents for acting against intracellular susceptible as well as multidrug resistant *S. aureus*.

**Results**

**Strains, MICs, resistance patterns and intracellular pharmacodynamic parameters**

- ATCC25923 (VAN S  DAP S  LZD S) was used as a reference strain.
- *S. aureus* ATCC25923 (VAN S  DAP S  LZD S) was used as a reference strain.
- **Table 1**: Strains, MICs, resistance patterns and intracellular pharmacodynamic parameters.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antibiotic MIC (mg/L)</th>
<th>EUCAST categor.</th>
<th>mg/L</th>
<th>xMIC</th>
<th>Emax (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATCC25923</td>
<td>Debio 1452</td>
<td>0.004</td>
<td>nd</td>
<td>4.8</td>
<td>0.89 (0.69 to 1.2)</td>
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<tr>
<td>MU50</td>
<td>Daptomycin</td>
<td>0.2</td>
<td>S</td>
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<tr>
<td>MU50</td>
<td>Vancomycin</td>
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<td>S</td>
<td>15.5 (13.5 to 17.6)</td>
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</tr>
<tr>
<td>SA404</td>
<td>LZD</td>
<td>2</td>
<td>S</td>
<td>15.5 (13.5 to 17.6)</td>
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</tr>
<tr>
<td>NRS119</td>
<td>Linezolid</td>
<td>64</td>
<td>S</td>
<td>15.5 (13.5 to 17.6)</td>
<td></td>
</tr>
</tbody>
</table>

**Figure 3**: Concentration-responses by strain and antibiotic

- Very high potency of Debio 1452 with MICs of 0.004 mg/L and C<sub>50</sub> between 0.01 and 0.03 mg/L in broth or intracellularly against all the strains used in this study (C<sub>50</sub> average: about 1,300 fold lower than the comparators for susceptible strains).
- An intracellular maximal efficacy (E<sub>max</sub>) similar to that of other drugs tested (-0.4 and -0.7 log<sub>10</sub> CFU decrease).
- No apparent effect of resistance mechanisms to other antibiotics in broth or intracellularly for the strains used.