Activities of Daptomycin (DAP), Vancomycin (VAN) and Linezolid (LDZ) alone or in combination with Fusidic acid (FUS) in an in vitro dynamic model of Staphylococcus aureus Biofilm

Wafi Siala,1 Prabhavathi Fernandes,2 Paul M. Tulkens,1 and Françoise Van Bambeke1

1. Pharmacomologie cellulaire et moléculaire, Louvain Drug Research Institute, Université catholique de Louvain, Brussels, Belgium; 2. Cempra, Inc., Chapel Hill, NC, USA

Poster A492

Abstract

Objective: FUS is currently evaluated as an oral drug for the treatment of cSSSTI in which biofilms play a major role. We evaluated the activity of FUS alone or combined with other antistaphylococcal antibiotics (DAP, VAN, LDZ) in an in vitro pharmacodynamic model of staphylococcal biofilm using the CDC reactor system, exposing biofilms to shear forces and mimicking antibiotic pharmacokinetics.

Methods: Biofilms of S. aureus ATCC25923 were grown at 37°C on polycarbonate coupons inserted into rods contained in the CDC biofilm reactor using a starting inoculum of 10^5 CFU/ml. Preconditioning was achieved in TSB + 1% glucose and 2% NaCl by 6h batch incubation followed by 14h of continuous flow (11.6 mL/min). Antibiotics were then injected at concentrations corresponding to their minimal inhibitory concentration (MIC) with flow rates adapted to simulate their respective half-lives. Coupons were collected over time and washed twice in PBS. Bacteria were recovered by 3 alternating 60 sec cycles of vortexing and sonication, and plated for CFU counting.

Results: FUS alone had no activity while VAN and DAP alone caused a minimal decrease in CFU (0.5-0.7 log). Combinations of FUS with DAP or with LZD were highly synergistic, reducing 2.45 and 3.97 log CFU decrease compared to control, respectively. In contrast, combining FUS with VAN did not markedly improve activity on biofilms.

Conclusion: Combinations of FUS with DAP or LZD were the most effective against S. aureus biofilm in this pharmacodynamic model, warranting testing in vivo.

Introduction

Staphylococcus aureus is an important human pathogen causing chronic infections that are difficult to treat. Biofilms contribute to persistence of infections, by protecting bacteria from the immune system and antimicrobial agents. We showed that many antibiotics are poorly active against biofilms, especially with resistance selection, our aim was to evaluate bacterial killing of biofilms [1]. Fusidic acid (FUS) may constitute a useful alternative for treatment of S. aureus infections [2].

Materials and Methods

Biofilms of S. aureus ATCC25923 were grown on polycarbonate coupons inserted into rods within the CDC biofilm reactor (BioSurfaces Technologies, Bozeman, MT). Antibiotics were preconditioned during 20h as follows:

a) 6h of incubation at 37°C of 10^5 CFU/ml in TSB + 1% glucose and 2% NaCl.
b) 14h of continuous flow at a rate of 11.6 ml/min.

Results

Reduction in log_{10} CFU/ml within biofilms for antibiotics alone or FUS combined with VAN, DAP, LZD compared to untreated biofilm of ATCC25923 strain

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>FUS alone</th>
<th>+ FUS alone + VAN</th>
<th>+ FUS alone + DAP</th>
<th>+ FUS alone + LZD</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>NE b</td>
<td>0.07 ± 0.02</td>
<td>0.05 ± 0.02</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.16 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>NE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.20 ± 0.03</td>
<td>0.02 ± 0.00</td>
<td>0.78 ± 0.17</td>
</tr>
<tr>
<td>4</td>
<td>0.04 ± 0.01</td>
<td>0.01 ± 0.01</td>
<td>0.38 ± 0.09</td>
<td>1.49 ± 0.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.10 ± 0.00</td>
<td>0.27 ± 0.1</td>
<td>3.27 ± 0.4</td>
</tr>
<tr>
<td>8</td>
<td>NE</td>
<td>0.37 ± 0.01</td>
<td>0.63 ± 0.01</td>
<td>1.79 ± 0.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.42 ± 0.1</td>
<td>0.53 ± 0.02</td>
<td>3.97 ± 0.23**</td>
</tr>
</tbody>
</table>

Fusidic acid (FUS) MIC: 0.5 mg/L; Daptomycin (DAP) MIC: 0.5 mg/L; Linezolid (LZD) MIC: 1 mg/L; Vancomycin (VAN) MIC: 1 mg/L.

FUS alone was not active on biofilms in this model.

FUS alone was not active on biofilms in this model.

The other antibiotics alone had reduced CFUs of only about 0.5 log_{10} at 18 h.

Combining FUS with VAN did not markedly improve its activity on biofilms.

In contrast, combining FUS with DAP or with LZD was highly synergistic, reducing bacterial counts by 2.5 and 4 log_{10} CFU, respectively.

References


Acknowledgements

This poster will be available after the meeting at http://www.facm.ucl.ac.be/posters

Conclusions and Future Directions

- Biofilms of S. aureus ATCC25923 were grown on polycarbonate coupons inserted into rods within the CDC biofilm reactor (BioSurfaces Technologies, Bozeman, MT).
- Biofilms were preconditioned during 20h as follows:
  - a) 6h of incubation at 37°C of 10^5 CFU/ml in TSB + 1% glucose and 2% NaCl.
  - b) 14h of continuous flow at a rate of 11.6 ml/min.
- Antibiotics were added in the bioreactor at their MIC with subsequent flow rate adapted to simulate the half-lives of the antibiotics.
- Coupons were aseptically removed at 0, 2, 4, 8, 12 and 18h and washed twice in PBS.
- Bacteria were recovered from biofilms by 3 alternating 60 sec cycles of vortexing and sonication.
- Samples were then serially diluted and plated onto TSA to allow colony counting.

This poster will be available after the meeting at http://www.facm.ucl.ac.be/posters

Poster A492

Materials and Methods

- Biofilms of S. aureus ATCC25923 were grown on polycarbonate coupons inserted into rods within the CDC biofilm reactor (BioSurfaces Technologies, Bozeman, MT).
- Biofilms were preconditioned during 20h as follows:
  - a) 6h of incubation at 37°C of 10^5 CFU/ml in TSB + 1% glucose and 2% NaCl.
  - b) 14h of continuous flow at a rate of 11.6 ml/min.
- Antibiotics were added in the bioreactor at their MIC with subsequent flow rate adapted to simulate the half-lives of the antibiotics.
- Coupons were aseptically removed at 0, 2, 4, 8, 12 and 18h and washed twice in PBS.
- Bacteria were recovered from biofilms by 3 alternating 60 sec cycles of vortexing and sonication.
- Samples were then serially diluted and plated onto TSA to allow colony counting.

Conclusions and Future Directions

- Biofilms of S. aureus ATCC25923 were grown on polycarbonate coupons inserted into rods within the CDC biofilm reactor (BioSurfaces Technologies, Bozeman, MT).
- Biofilms were preconditioned during 20h as follows:
  - a) 6h of incubation at 37°C of 10^5 CFU/ml in TSB + 1% glucose and 2% NaCl.
  - b) 14h of continuous flow at a rate of 11.6 ml/min.
- Antibiotics were added in the bioreactor at their MIC with subsequent flow rate adapted to simulate the half-lives of the antibiotics.
- Coupons were aseptically removed at 0, 2, 4, 8, 12 and 18h and washed twice in PBS.
- Bacteria were recovered from biofilms by 3 alternating 60 sec cycles of vortexing and sonication.
- Samples were then serially diluted and plated onto TSA to allow colony counting.

References


This poster will be available after the meeting at http://www.facm.ucl.ac.be/posters