Determination of the effects of a novel antimicrobial agent used in conjunction with Gentamicin on Staphylococcus aureus using a porcine model: preliminary evaluations, Florida

Abstract:
The presence of wound infections, especially associated with Staphylococcus aureus, is a major concern for healthcare providers. S. aureus is one of the most common pathogens found in chronic wounds. Furthermore, S. aureus readily forms biofilms, which surface-adherent bacterial communities that render the bacteria resistant to antibiotics and host immune responses, and greatly increase healthcare treatment costs. Therefore, novel strategies that reduce the biofilm phenotype of S. aureus are highly desirable. Our study aimed to test the ability of DTPA to inhibit biofilm formation of S. aureus along with a common topical antibiotic, Gentamicin.

Introduction:
S. aureus readily forms biofilms, which are characterized by the intercellular adhesion of bacteria. By introducing DTPA into the environment of S. aureus, the chelator can bind zinc, which is essential for biofilm formation. The ability of DTPA to inhibit biofilm formation can be attributed to the chelation of zinc, which is an essential component of the intercellular adhesion module.

Materials and Methods:
All experiments were conducted using S. aureus ATCC 29213. The bacterial strain was grown in Luria-Bertani broth at 37°C for 18 hours. The bacterial culture was then collected, washed, and suspended in saline solution to achieve a concentration of 10^8 CFU/ml. The bacterial suspension was then used to inoculate the wounds.

Animal Wounding:
All experiments were performed on a porcine model. Four wounds of 10mm x 7mm x 0.5mm were created on the paravertebral area on two pigs. The wounds were then treated with the appropriate treatment groups as described below.

Incubation:
A 28-day incubation period of 10^6 CFU/ml was inoculated into each wound, as seen in the image below.

Experimental Design:
Fig 1: Treatment Groups
A. 2500 µM DTPA
B. 5000 µM DTPA
C. 10000 µM DTPA
D. 30000 µM DTPA + Gentamicin 0.1%
E. 300 µM DTPA
F. 100 µM DTPA + Gentamicin 0.1%
G. 5000 µM DTPA + Gentamicin 0.1%
H. 10000 µM DTPA + Gentamicin 0.1%
I. Vehicle
J. Untreated

Fig 2: Treatment Groups
A. 2500 µM DTPA
B. 5000 µM DTPA
C. 10000 µM DTPA
D. 30000 µM DTPA + Gentamicin 0.1%
E. 300 µM DTPA
F. 100 µM DTPA + Gentamicin 0.1%
G. 5000 µM DTPA + Gentamicin 0.1%
H. 10000 µM DTPA + Gentamicin 0.1%
I. Vehicle
J. Untreated

Experimental Wound Site Treatment:

<table>
<thead>
<tr>
<th>Wound Site</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig 1: Treatment Groups</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>2500 µM DTPA</td>
</tr>
<tr>
<td>B</td>
<td>5000 µM DTPA</td>
</tr>
<tr>
<td>C</td>
<td>10000 µM DTPA</td>
</tr>
<tr>
<td>D</td>
<td>30000 µM DTPA + Gentamicin 0.1%</td>
</tr>
<tr>
<td>E</td>
<td>300 µM DTPA</td>
</tr>
<tr>
<td>F</td>
<td>100 µM DTPA + Gentamicin 0.1%</td>
</tr>
<tr>
<td>G</td>
<td>5000 µM DTPA + Gentamicin 0.1%</td>
</tr>
<tr>
<td>H</td>
<td>10000 µM DTPA + Gentamicin 0.1%</td>
</tr>
<tr>
<td>I</td>
<td>Vehicle</td>
</tr>
<tr>
<td>J</td>
<td>Untreated</td>
</tr>
</tbody>
</table>

Pig 2: Treatment Groups
<table>
<thead>
<tr>
<th>Wound Site</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>2500 µM DTPA</td>
</tr>
<tr>
<td>B</td>
<td>5000 µM DTPA</td>
</tr>
<tr>
<td>C</td>
<td>10000 µM DTPA</td>
</tr>
<tr>
<td>D</td>
<td>30000 µM DTPA + Gentamicin 0.1%</td>
</tr>
<tr>
<td>E</td>
<td>300 µM DTPA</td>
</tr>
<tr>
<td>F</td>
<td>100 µM DTPA + Gentamicin 0.1%</td>
</tr>
<tr>
<td>G</td>
<td>5000 µM DTPA + Gentamicin 0.1%</td>
</tr>
<tr>
<td>H</td>
<td>10000 µM DTPA + Gentamicin 0.1%</td>
</tr>
<tr>
<td>I</td>
<td>Vehicle</td>
</tr>
<tr>
<td>J</td>
<td>Untreated</td>
</tr>
</tbody>
</table>

Treatment:
• Within 20 minutes of inoculation each wound was treated with the appropriate combination of the antimicrobial agent.
• Each wound was treated with 200µl of antimicrobial agent.
• All wounds were covered with polyurethane film dressings.

Results:
• The wounds treated with 30000 µl DTPA contained the lowest counts of S. aureus compared to all other concentrations of DTPA.
• Treatment with this concentration resulted in a 99.96% reduction in bacterial load compared to the untreated group.
• Increases in DTPA concentration were directly proportional to the increases in the percentage of both planktonic and biofilm-associated bacteria.
• All wounds, with the exception of those treated with 30 µM DTPA, contained more bacteria in the biofilm phenotype than in a planktonic state.
• The 0.1% Gentamicin positive control produced substantially more reduction in wound bioburden than any of the DTPA concentrations alone.

Conclusion:
• The increased reduction of S. aureus wounds was directly proportional to the increased concentration of DTPA with Gentamicin.
• The 3000 µl concentration of DTPA used produced a planktonic bacterial count of 1.5±2.0 Log CFU/ml and a biofilm of 6.9±2.1 Log CFU/ml, whereas 3000 µM DTPA in conjunction with 0.1% Gentamicin reduced wound counts containing planktonic and biofilm bacterial levels below the level of quantification (1.3 Log CFU/ml). These were over a 38.9±2.0 and 7.4±1.2 Log reductions in colony forming units in planktonic and biofilm bacteria, respectively.
• These data suggest that the zinc chelator, DTPA, has an antimicrobial effect that is synergistic when used in combination with Gentamicin.
• Additional studies on the effectiveness of DTPA with other antibiotics to reduce infections are warranted.

References:

Acknowledgements:
This research was supported by grants from the US Department of Defense (DAMD17-02-1-0601) and the National Institute of Biomedical Imaging and Bioengineering (5R01EB000247-02). We would like to thank Dr. Sarah Riggs, Dr. Lisa A. Cazaniga, Dr. Edward Welch, and Dr. Daniel C. Kurtz for their valuable contributions to this project. We also thank Dr. Michael J. Muzzarelli for his help in writing this manuscript.