Cationic Antimicrobial Peptide LL-37 Disrupts Staphylococcus Aureus Biofilms

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Disclosures:

Introduction: Biofilms are often found on biomedical devices and are very difficult to eliminate; no effective approach has been reported. Our previous studies have shown that LL-37, a cationic antimicrobial peptide (CAMP) and a component of the innate immune system, has high potency against both intra- and extra-cellular Staphylococcus aureus (S. aureus). Unlike conventional antibiotics, amphiphilic CAMPs like cathelicidin LL-37 are comprised of hydrophobic and hydrophilic residues aligned on opposite sides of the peptide, which may facilitate their penetration through biofilms to disrupt them. In this study, our aim was to establish biofilms using S. aureus and to determine the effectiveness of LL-37 in disrupting the biofilms. We hypothesized that LL-37 would be effective in disrupting S. aureus biofilms.

Methods: Five S. aureus strains were examined including bioluminescent strains (Xen36, Xen29, Xen8.1), ATCC25923, and clinical 1004. Four sets of experiments were carried out: (i) Optimizing bacterial concentration in establishing biofilms using S. aureus Xen36. (ii) Establishing biofilms using the five bacterial strains and correlating bioluminescent intensity of the bioluminescent strains with colony forming units (CFUs) at different time points. (iii) Examining the effect of LL-37 concentrations in disrupting biofilms. (iv) Comparing the effect of LL-37 with conventional antibiotics in disrupting S. aureus biofilms. In more details, Xen 36 was first characterized using three different methods. The bacteria were grown in 24-well plates on stainless steel discs at three different starting concentrations: 10^2, 10^4, and 10^6 CFU/mL. Biofilms were grown on stainless steel discs of 1-cm diameter for specified times: 4, 8, 12, 24, 72, 120, 168, and 504 hr. At each time point, discs were removed for IVIS imaging, optical density measurement, and CFU counting. Next, the five bacterial strains (Xen36, Xen29, Xen8.1, ATCC25923, and clinical 1004) were grown on stainless steel discs at an initial concentration of 10^2 CFU/mL. Biofilms of these strains were grown to time points of 4, 8, 12, 24, 48, 168, 336, and 504 hr, and then the discs were analyzed using IVIS imaging, optical density measurement, and CFU counting. Scanning electron microscopy (SEM) was also used to examine biofilms at time points 4, 24, 48, 168, and 504 hr. Subsequently, the biofilms were treated with LL-37 and antibiotics: Biofilms were grown on stainless steel discs for 24 hr in an incubator on a rocker using Xen36, ATCC25923, and clinical 1004. They were then treated at room temperature using 100 µM concentration of LL-37, lactoferricin B, clindamycin, vancomycin, or cefazolin. After 24 hr, the discs were washed with phosphate buffered saline (PBS) and processed for CFU, IVIS, and SEM imaging. Discs were sonicated in PBS and plated on LB agar plates for the bioluminescent strains and on blood agar plates for the clinical and ATCC strains. CFUs were enumerated using an Acolyte colony counter.

Results: We found that a concentration of 10^2 CFU/mL of S. aureus Xen36 could provide detectable bioluminescent intensity, and the growth profiles of 10^2, 10^4, and 10^6 CFU/mL had similar patterns at the time points studied (data not shown). The biofilm-forming capacity of the various S. aureus strains were observed by cultivating the biofilms on polystyrene and stainless steel surfaces. We found that all S. aureus strains examined formed biofilms but their biofilms had very different morphologies (Fig. 1); biofilms formed quicker on polystyrene surfaces compared to stainless steel ones (data not shown). The capacity of LL-37 to disrupt S. aureus biofilms was determined and we found that LL-37 was highly potent in the disruption of S. aureus biofilms compared to the other CAMP (i.e. lactoferricin-B) and the conventional antibiotics such as clindamycin, vancomycin, and cefazolin, and LL-37 reduced the CFUs more than 70% in the clinical 1004, ATCC25923, and Xen36 S. aureus strains (Fig. 2).

Discussion: Our previous studies have demonstrated that LL-37, compared to conventional antibiotics, is more potent and faster at eliminating both extra- and intra-cellular S. aureus. LL-37 was also found to exhibit synergistic antibacterial activities with β-defensin and lysozyme in both neutral and acidic environments. However, it was unknown whether LL-37 would be more effective in disrupting Staphylococcal biofilms compared to conventional antibiotics. We have now demonstrated in vitro that LL-37 could disrupt S. aureus biofilms and that it was significantly more effective in disrupting S. aureus biofilms compared to commonly used conventional antibiotics (e.g. clindamycin, vancomycin, and cefazolin). The disruption of S. aureus biofilms was believed to take place through the lysis of the Staphylococci which leads to destabilization of the biofilm matrix. Administration of LL-37 may disrupt Staphylococcal biofilms and therefore may have the potential to eliminate the need for surgical removal of infected biomedical devices. In brief, we found that Xen36 was stable and had a high in vitro bioluminescent signal, biofilms were developed on stainless steel discs with the bioluminescent bacteria, and LL-37 disrupted the bioluminescent biofilms and was much more effective compared to commonly used conventional antibiotics (e.g. clindamycin, vancomycin, and cefazolin).

Significance: S. aureus has been commonly found to grow biofilms on biomedical devices and has been a significant clinical concern. Unfortunately, very few therapeutic approaches have been reported as effective in disrupting or eliminating such biofilms. In this study, we found that LL-37 was much more effective in disrupting S. aureus biofilms compared to conventional antibiotics and could potentially contribute to biofilm removal clinically.
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