Cationic Antimicrobial Peptides Potent against Common Orthopedic Pathogens

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Statement of Purpose: Surgical site infection is a significant clinical complication of a major operation, affecting literally millions of patients annually. The Centers for Disease Control and Prevention (CDC) estimates that ~290,000 surgical site infections occur annually in the U.S., resulting in more than $1 billion to $10 billion in direct and indirect medical costs to the U.S. healthcare. Approximately 8,000 patient deaths a year are associated with these infections. Also, the CDC estimates that the rates of surgical site infection range from 2-5% for clean cases, and may be higher (~20%) depending on procedure type, patient case-mix, and wound type (contaminated) among other factors. 33% of surgical site infections are orthopedic infections. However, the heavy use of antibiotics to treat surgical site infections has caused bacteria to mutate and emerge as multi-drug resistant "super bugs" such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *S. aureus* (VRSA). In 2009, the CDC reported a sharp increase in the number of severe bacterial infections especially caused by *Staphylococcus*.

Compared to conventional antibiotics, cationic antimicrobial peptides (CAMPs) have the potential to be more potent in killing pathogens and less likely to induce bacterial resistance. Here we report the efficacy of CAMPs against conventional antibiotics on *S. aureus*, one major culprit of orthopedic infections.

Methods: A clinical isolate of *S. aureus* from a patient's chronic wound was obtained and treated in its Log phase (exponential bacterial growth) with CAMPs and conventional antibiotics. Cathelicidin LL-37, Lactoferricin-B, Doxycycline, and Cefazolin were added to the bacterial media for 30 min at various molar concentrations ranging from 10 nm to 100 mM under the same experimental conditions. LL- 37 was tested on an American Type Culture Collection (ATCC) strain (25923) of *S. aureus* at 1.0 μM, 2.0 μM, and 3.0 μM concentrations for comparison. Zone of inhibition (ZOI) and kinetic studies were also conducted.

Results: CAMPs including Cathelicidin LL-37 and Lactoferricin-B were found to be more potent than conventional antibiotics (e.g., Doxycycline and Cefazolin) in eliminating *S. aureus* (**Figure 1**). LL-37 was potent in killing over 90% of the bacteria even at very low molar concentrations (250 nM or lower). Lactoferricin-B exhibited over 90% killing at 25.0 μM concentration whereas Doxycycline and Cefazolin had a similar killing potency at 1.0 mM. Moreover, LL-37 showed much faster kinetics in killing *S. aureus* (data not shown). In addition, LL-37 exhibited a phase specific response (data not shown) with a higher killing ability toward the ATCC strain at the Stationary phase than at the Log phase at 1.0 μM concentration. It also exhibited a strain specific rapid elimination (data not shown) of the clinical strain compared to the ATCC strain especially at 1.0 μM concentration. The ZOI data were consistent with these findings (data not shown).

Conclusions: We found that CAMPs like LL-37 were very potent and quick in killing *S. aureus*. LL-37 was 100 times more potent than Lactoferricin-B and 4,000 times more potent than Doxycycline and Cefazolin. LL-37 could therefore be a good candidate for treating surgical site infections and has the potential to reduce the use of antibiotics; LL-37 was potent in killing *S. aureus* at nano-molar levels. In future studies, the *in vivo* efficacy of LL-37 against *S. aureus* infection will be tested in our open fracture rat model.

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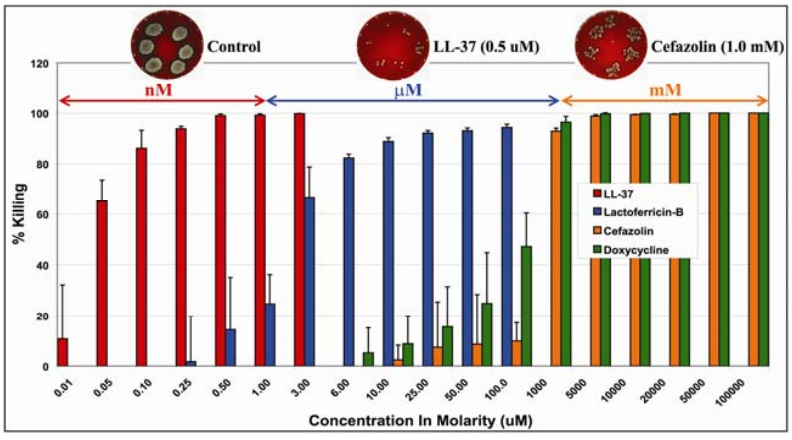

Figure 1. Killing potencies of CAMPs and conventional antibiotics. LL-37 is effective in killing *S. aureus* at nM concentrations, while Lactoferricin-B at μM concentrations and Doxycycline and Cefazolin at mM concentrations.