Antimicrobial Peptide Presents Potent Antibacterial Properties and Low Toxicity toward Human Cells

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INTRODUCTION: In surgery, including orthopaedic surgery, preventing infections is a major concern that plays an important role in clinical outcomes. The broad use of implants among various surgeries has significantly increased the occurrence of infections, while the globally abuse of antibiotics has led to bacterial antibiotic resistance that has resulted in the rise of 'superbugs' like methicillin resistant *Staphylococcus aureus* (MRSA). There is an urgent need for treatment modalities that can be used to substitute antibiotics. Antimicrobial peptides (AMPs), existing in a variety of forms in animals, have emerged as an alternative to conventional antibiotics since the 1980s. Natural AMPs have common characteristics including being relatively short (30 to 60 amino acids), cationic (+2 to +9), and amphiphilic (>50% hydrophobic amino acids). AMPs could be used as immunomodulatory molecules to regulate the body's immune function, change the host immune related gene expression, suppress the inflammatory cytokines induced by lipopolysaccharides, promote wound healing, enhance angiogenesis, and play a bridge role for the mononuclear cells, macrophages, and dendritic cells in innate and acquired immune responses. However, most AMPs like LL-37 also present high toxicity toward human cells. In this study, an AMP with a nine-amino acid sequence of KRWWKWWRR (referred to as HHC36) was synthesized and tested *in vitro* against bacteria and mammalian cells. We hypothesized that this HHC36 has high antimicrobial activity against bacteria like *Staphylococcus aureus* (*S. aureus*) and low toxicity toward mammalian cells like osteoblasts.

METHODS: The peptide (i.e., HHC36) was synthesized by CPC Scientific Inc. (Sunnyvale, CA) and its sequence was Lys-Arg-Trp-Trp-Lys-Trp-Trp-Arg-Arg-NH_2 with a MW of 1,487.8 and a purity of 98.2%. The antimicrobial properties of HHC36 were assessed against *S. aureus*, and cytotoxicity toward mammalian cells was evaluated using human osteoblast and BEAS-2B cells and compared with conventional antibiotics (gentamicin, rifampin, vancomycin, cefazolin, and fusidic acid). Statistical analysis was carried out using JMP-V9 statistical software and $p<0.05$ was considered statistically significant.

RESULTS: The antimicrobial activity of HHC36 was dose dependent. After 30 min, the killing percent of HHC36 against *S. aureus* was about 15% at 3 μM, and increased sharply and significantly to approximately 50% at 30 μM and over 90% at 100 μM; 100% bacterial killing was achieved at the concentration of 200 μM and above (**Fig. 1a**). It was found that conventional antibiotics like vancomycin, rifampin, cefazolin, and fusidic acid all had significantly lower bacterial killing percent compared to HHC36 at the same concentration (i.e., 200 μM) after culturing for 30 min (**Fig. 1b**). HHC36 had 100% killing, while fusidic acid and vancomycin presented only about 20% killing of *S. aureus*; rifampin and cefazolin had about 40% and 60% bacterial killing, respectively. Like HHC36, gentamicin also achieved 100% bacterial killing (**Fig. 1b**); however, gentamicin showed much slower kinetics compared to HHC36, since HHC36 eliminated more than 90% of *S. aureus* within 5 min when gentamycin only had about 50% bacterial killing at 5 min (**Fig. 1c**). Cytotoxicity studies found that HHC36 had significantly higer (30% higher) osteoblast cell viability compared to the treatments of all conventional antibiotics studied at 2 h (**Fig. 1d**). The toxicity toward human cells (i.e., osteoblasts and BEAS-2B cells) was dose dependent; the higher the concentration the lower the cell viability (**Fig. 1e**).

DISCUSSION: AMPs can present their antibacterial activities for different targets, the most obvious of which is the targeting of the membrane structure. Bacterial membranes are negatively charged and AMPs tend to selectively attack bacteria more compared to mammalian cells due to the cationic characteristics of AMPs. It is reasonable, though not well understood, that the toxic effects of AMPs are peptide sequence or chemical structure dependent and AMPs with certain amino acid sequences may have limited toxicity toward mammalian cells. In this study, HHC36 was synthesized and tested. The data showed that, compared with commonly used conventional antibiotics, HHC36 was effective and prompt at eliminating bacteria like *S. aureus*. It presented significantly higher osteoblast cell viability compared to the conventional antibiotics studied. Therefore, HHC36 may have the potential to be a better alternative to the current antibiotics, although further studies are warranted.

SIGNIFICANCE: During the history of antimicrobial agents, there is a challenge that has not been addressed where most, if not all, antimicrobial agents have resulted in reduced host cell viability thereby compromising host responses. In the field of orthopaedics, such an issue may be reflected as the inhibition of osteogenesis, which, for instance, may lead to delayed union or non-union of fractures. Therefore, identifying and developing antimicrobial agents that not only achieve strong antimicrobial properties but also present minimal toxicity toward mammalian cells is clinically important.

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IMAGES AND TABLES:

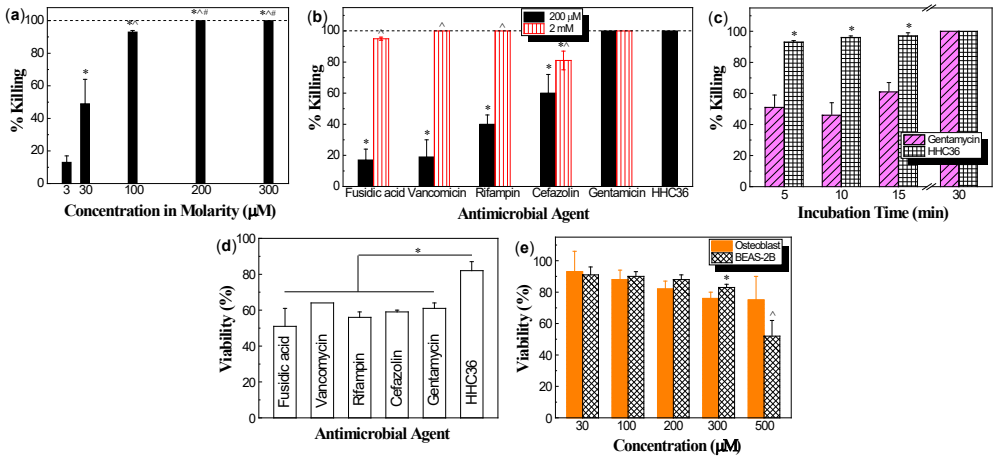

Fig. 1. (**a**) Killing percent of *S. aureus* by HHC36 at 30 min. *$p<0.05$ compared to 3 μM; ^$p<0.05$ compared to 30 μM; and #$p<0.05$ compared to 100 μM. (**b**) Killing percent of *S. aureus* by HHC36 and conventional antibiotics for 30 min. *$p<0.05$ compared to HHC36; ^$p<0.05$ compared to 200 μM of the same antibiotic. (**c**) Bacterial killing kinetics against *S. aureus* at 200 μM. *$p<0.05$ compared to gentamycin at the same treatment time. (**d**) Effects of different antimicrobial agents (200 μM) on osteoblast viability at 2 h. *$p<0.05$ compared to all antibiotic treatments. (**e**) Viability of osteoblasts and BEAS-2B cells for HHC36 of various concentrations at 2 h. *$p<0.05$ compared to osteoblast at 300 μM; ^$p<0.05$ compared to BEAS-2B at 30, 100, 200, and 300 μM.