Antimicrobial Peptide Kills Bacteria but Presents Low Toxicity toward Human Cells

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Statement of Purpose: Implant associated infections have been significant clinical issues and currently, antibiotic treatment is primarily used to prevent or treat such infections. However, the heavy use of antibiotics has caused bacteria to mutate and emerge as multi-drug resistant "super bugs" such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA). In time, without the development of new and effective antimicrobial drugs, it is possible that certain pathogens will be untreatable by conventional antibiotics. To deal with this antibiotic resistance concern, a variety of cationic antimicrobial peptides have been reported in the literature. However, the current cationic antimicrobial peptides are highly toxic to human cells as well as to bacteria and their toxicity (toward human cells) has been the biggest barrier for their clinical applications. The *objective* of this study was to examine *in vitro* the antimicrobial and toxicity properties of a new synthetic peptide. We *hypothesized* that the synthetic peptide to be studied has significantly higher antimicrobial properties but significantly lower toxicity toward human cells compared to conventional antibiotics.

Methods: A new synthetic peptide (designated as Pep-L_{cyto}) was designed, synthesized, and purified; its purity was 98.2% as determined by high-performance liquid chromatography. Conventional antibiotics (fusidic acid, cefazolin, rifampin, and vancomycin) were purchased from Sigma-Aldrich. The antimicrobial effect of the synthetic peptide and antibiotics were tested via a survival assay by counting the colony forming units (CFUs) in triplicate experiments. A clinical strain of *Staphylococcus aureus* (*S. aureus*), obtained from a patient's chronic wound, was studied because *S. aureus* is one of the major pathogens responsible for implant associated infections. The effect of the synthetic peptide and antibiotics on the viability of human osteoblast cells (ATCC CRL-11226) and BEAS-2B lung epithelial cells (ATCC CRL-9609) were examined via an MTT cell viability assay. The outcomes were presented as percentages compared to controls without peptide and antibiotic treatments. Statistical analyses were carried out using JMP-V9 statistical visualization software and p values < 0.05 were considered significant.

Results: The synthetic peptide (i.e. Pep-L_{cyto}) was found to be potent and quick in killing *S. aureus*. Its bacterial killing percentage increased with increasing peptide concentration and achieved 100% killing at 200 µM within 30 min (Fig. 1a). The bacterial killing of Pep-L_{cyto} was also fast: ~93% *S. aureus* was killed within the first 5 min (Fig. 1a inset). Meanwhile, compared to commonly used antibiotics (e.g. vancomycin, rifampin, cefazolin, and fusidic acid), Pep-L_{cyto} showed significantly higher killing efficacy toward bacteria like *S. aureus* while exhibiting significantly lower toxicity toward human cells like osteoblasts (Fig. 1b). One can see that Pep-L_{cyto} at 200 µM maintained above 80% viability of osteoblasts with a 100% bacterial killing (Fig. 1b). Similarly, Pep-L_{cyto} at 200 µM and below presented high viability (~90%) of BEAS-2B cells (data not shown).

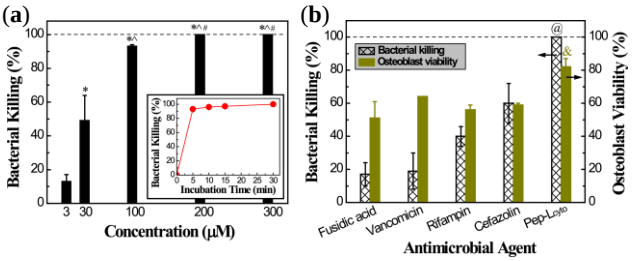

Figure 1. (a) Bacterial killing percentage of Pep-L_{cyto} at various concentrations; inset is the kinetics at 200 µM. The *S. aureus* inoculum was 3×10^5 CFU/mL and the incubation time was 30 min. $^*p<0.05$ compared to 3 µM, $^\wedge p<0.05$ compared to 30 µM, and $^\#p<0.05$ compared to 100 µM. **(b)** Bacterial killing percentage and osteoblast viability of Pep-L_{cyto} and multiple commonly used antibiotics against *S. aureus* at 200 µM; incubation time for bacterial killing tests was 30 min; osteoblast viability testing was 2 hrs. The *S. aureus* inoculum was 3×10^5 CFU/mL and the osteoblast used was 3×10^4/mL. $^{@,\&}p<0.05$ compared to fusidic acid, vancomycin, rifampin, and cefazolin.

Discussion: Cationic antimicrobial peptides are considered an important part of the host defense system against microorganisms and a variety of cationic antimicrobial peptides have been studied. Compared to conventional antibiotics, cationic antimicrobial peptides are less likely to provoke microbial resistance. However, the current cationic antimicrobial peptides, to our best knowledge, are highly toxic to human cells as well as to bacteria and their toxicity (toward human cells) has been the biggest barrier for their clinical application. The synthetic peptide reported here had significantly higher antimicrobial properties against *S. aureus* along with significantly reduced toxicity toward human cells like osteoblasts and BEAS-2B cells compared to commonly used antibiotics.

Conclusions: A new synthetic peptide was demonstrated *in vitro* to have significantly higher antimicrobial properties along with significantly lower toxicity toward human osteoblasts and lung epithelial cells compared to conventional antibiotics. Such an antimicrobial peptide may lead to significant beneficial outcomes (e.g. reduced infection and improved healing) in treating implant associated infections, especially those induced by antibiotic resistant bacteria. In future studies, the antimicrobial and toxicity properties of this synthetic peptide may be evaluated in an open fracture rat infection model.[1,2]

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References:

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[2]. Li B, *et al.* J Orthop Res 2010;28(1):48-54.