Cationic Self-Assembling Peptide Amphiphiles as Antibacterial Agents Against Drug-Resistant Bacteria

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Introduction: Bacterial antibiotic resistance has become one of the biggest threats to global health. In the United States alone, at least 2 million cases of illness and 23,000 of deaths are caused by antibiotic-resistant bacteria annually. The overuse of antibiotics is the most important factor responsible to the development of antibiotic resistance around the world [1]. To overcome bacterial drug resistance and to reduce the use of antibiotics, many nanoscale antibacterial agents have been developed to kill bacteria by disrupting the bacterial cell membrane. In this study, we have designed and developed a type of self-assembling antibacterial peptide amphiphile that can kill drug-resistant bacteria without the use of antibiotics.

To develop a self-assembling peptide molecule of interest, the antibacterial cationic peptide amphiphiles (ACA-PAs) were rationally designed to self-assemble into a cylindrical supramolecular structure in solution via non-covalent interactions. These ACA-PAs exhibit antibacterial properties similar to those of cationic antimicrobial peptides (AMPs). Unlike antibiotics, cationic AMPs can disrupt bacterial cell membranes by electrostatic attachment and insertion; this activity can reduce likelihood for drug-resistance development as the target site of the peptide is the entire bacterial cell membrane [2]. For peptide self-assembly, each molecule of the self-assembling ACA-PAs contains: i) a hydrophobic tail group conjugated to the amine-end of the peptide; ii) a sequence of consecutive hydrophobic amino acids that forms β-sheet secondary structure with intermolecular hydrogen bonds; and iii) a cationic heparin-binding peptide sequence. In water, the hydrophobic interactions of the hydrocarbon tail group of the ACA-PAs serve as the driving force of self-assembly, and the β-sheet stacking directs the aggregated structure into cylindrical shape [3], which enables the formation of transmembrane pores on bacterial cell membranes [4]. As a result, the cationic polar heparin-binding group is exposed on the surface of the self-assembled structure to interact with anionic bacteria membranes.

Materials and Methods: Lyophilized ACA-PAs powder were synthesized by Biomatik (ON, Canada). The self-assembled structure of ACA-PAs were was prepared by dissolving the lyophilized powder in deionized water followed by overnight incubation at 4 ℃. Transmission electron microscope (TEM) and circular dichroism (CD) characterization were used to observe the structure. In order to evaluate the minimum peptide concentration required for self-assembly, the critical micelle concentration (CMC) of ACA-PAs was determined by the fluorescent enhancement of the lipophilic Nile Red dye. In bacterial studies, Gram-positive methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC#43360) and Gram-negative multidrug-resistant *Escherichia coli* (MDR *E.coli*, ATCC#BAA-2471) were used to test the antibacterial activity of ACA-PAs. Bacteria were incubated in the ACA-PAs at 20 to 100 μM in 3% tryptic soy broth (TSB) media at 37 °C, and the bacterial growth curve were monitored in terms of turbidity by the optical density at 562 nm (O.D. at 562 nm) for 20 hours. The toxicity of ACA-PAs to bacteria was determined by viable colony count assay and by fluorescent live/dead assay after the 4 h treatment with the ACA-PAs. To study the effects of ACA-PAs on bacterial cell membranes, the fixed and ultrathin-sectioned bacteria samples were characterized by TEM. The cytotoxicity of the ACA-PAs was determined from tests performed on human dermal fibroblast (HDF) cells using a MTS assay.

Results and Discussion: TEM characterization showed successful self-assembly of the ACA-PAs into nanorods with diameters about 10 nm (Figure 1A). The Nile Red assay indicated that ACA-PAs self-assembled at peptide concentrations over 45 μM. As the circular dichroism spectra showed, the self-assembly of these ACA nanorods was directed by the β-sheet secondary structure. In bacterial studies, the ACA-PAs showed significant growth inhibitory effects and toxicity against all the bacterial strains tested, but exhibited different antibacterial properties on Gram-positive and Gram-negative bacteria (Figures 1B & 1C). For Gram-positive bacteria, the ACA-PAs displayed concentration-dependent growth inhibition and toxicity, which delayed the bacterial growth to 15 h and induced a 100-fold reduction in bacterial viability. For Gram-negative bacteria, the antibacterial activity of self-assembling ACA-PAs was remarkably higher above the concentration required for self-assembly, resulting in no bacterial growth over 20 h. TEM characterization of bacteria showed that the ACA-PAs caused localized bacterial membrane damage and leakage of cytosols. Compared to the toxicity against bacteria, the ACA-PAs had less cytotoxicity to HDF cells, suggesting good selectivity of the ACA-PAs towards the bacteria.

Conclusion: This study showed that the self-assembling ACA-PAs can self-assemble into nanorods, and have excellent antibacterial effects against antibiotic-resistant bacteria. In particular, self-assembly of ACA-PAs is important for killing Gram-negative bacteria. These self-assembling peptides could be promising nanomedicines to combat infectious disease caused by bacterial drug-resistance.

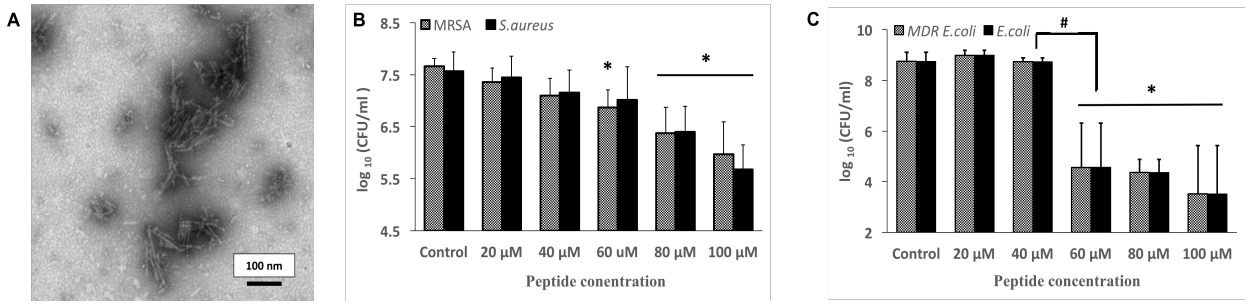

Figure 1: (A) TEM image showing the self-assembled nanorod structure of the antibacterial cationic peptide amphiphiles (ACA-PAs). The sample was prepared by dissolving the ACA-PAs in deionized water followed by negative staining before imaging; (B) evidence of the concentration-dependent bactericidal effect of ACA-PAs against Gram-positive methicillin-resistant *S.aureus* (MRSA) and *S.aureus.* The toxicity of ACA-PAs is enhanced proportionally with increased concentration but independent of peptide self-assembly and (C) enhanced bactericidal effect of ACA-PAs at concentrations over critical micelle concentration (CMC) (45 μM) against Gram-negative multidrug-resistant *E.coli (*MDR *E.coli*) and *E.coli*. Once self-assembled into nanorods over the CMC, the ACA-PAs induced remarkable toxicity towards Gram-negative bacteria. Results were expressed by standard error of the mean (S.E.M.). N=3, *p<0.005 compared with the control samples, #p,0.005 compared with samples treated by 40 μM of ACA-PAs.

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