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Novel chimeric peptide with enhanced cell specificity and anti-inflammatory activity

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ABSTRACT

An antimicrobial peptide (AMP), Hn-Mc, was designed by combining the N-terminus of HPA3NT3 and the C-terminus of melittin. This chimeric AMP exhibited potent antibacterial activity with low minimal inhibitory concentrations (MICs), ranging from 1 to 2 μM against four drug-susceptible bacteria and ten drug-resistant bacteria. Moreover, the hemolysis and cytotoxicity was reduced significantly compared to those of the parent peptides, highlighting its high cell selectivity. The morphological changes in the giant unilamellar vesicles and bacterial cell surfaces caused by the Hn-Mc peptide suggested that it killed the microbial cells by damaging the membrane envelope. An *in vivo* study also demonstrated the antibacterial activity of the Hn-Mc peptide in a mouse model infected with drug-resistant bacteria. In addition, the chimeric peptide inhibited the expression of lipopolysaccharide (LPS)-induced cytokines in RAW 264.7 cells by preventing the interaction between LPS and Toll-like receptors. These results suggest that this chimeric peptide is an antimicrobial and anti-inflammatory candidate as a pharmaceutic agent.

1. Introduction

The emergence of drug-resistant pathogens is due to the abuse and misuse of antibiotics in the biomedical fields. Drug-resistance is induced mostly by two mechanisms, inhibiting drug accumulation into the cytosol via multidrug efflux pumps [1–3] and phenotypic changes during infection, such as biofilms [4,5]. Until now, a number of studies have searched for new antibiotics with a different mechanism. Among them, antimicrobial peptides (AMPs), which are host defense molecules in a broad range of organisms, is a promising new antimicrobial candidate [6–8]. Although the sequences of AMPs vary, they have similar characters, such as a broad-spectrum of antimicrobial activity, amphipathicity, small length, cationic net charge, and rapid killing kinetics [9–13]. Their cellular targets are the plasma membrane and cytosolic components, but their mode of action in microbes is not completely understood [14,15]. Membranolytic AMPs induce alterations of the membrane potential or direct membrane disruption via "barrel-stave", "carpet", "toroidal", and "aggregation" models [16–21]. On the other hand, some AMPs are translocated to the cytoplasm of microbial cells via spontaneous lipid-assisted and receptor or channel-mediated translocation [15]. The precise mode of action in which AMPs enter spontaneously across the microbial membranes has not been determined. On the other hand, several models, such as the transient pore formation [22], lipid phase boundary defects [23] and the disordered toroidal pore formation [24,25], have been proposed. The internalized peptides inhibit or kill bacterial cells by inhibiting macromolecules synthesis, chaperone-mediated protein folding, cell wall synthesis, and cytoplasmic membrane septum formation. On the other hand, the uptake mechanism and cytoplasmic targets of many bacteria-penetrating peptides have not been demonstrated or yet undefined.

In the present study, the N-terminus of HPA3NT3 and C-terminus of melittin were collected to design a novel antimicrobial peptide with high antimicrobial activity and low cytotoxicity. HPA3NT3 is an analog peptide, derived from HP(2–20) with residues 2–20 of the parental HP from the *Helicobacter pylori* ribosomal protein L1. The peptide has potent antimicrobial activity through

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membranolytic action [26–28]. However, it is difficult to apply *in vivo* due to the cytotoxicity and aggregation at high concentrations. Melittin, from the European honeybee venom (*Apis mellifera*), is cytotoxic peptide with 26 amino acids and possesses a broad spectrum of antimicrobial activity [29,30]. Although its cytotoxicity and allergenicity are high, a number of studies have examined potential applications in therapeutic and biotechnological fields, focusing on bacterial infections [31], rheumatoid arthritis [32], arteriosclerosis [33], immunologic adjuvants [32], cancer [34], and endosomolytic material in drug delivery systems [35,36]. The substitution and deletion of amino acids are general methods to reduce the cytotoxic effect, but it is sometimes limited due to the length and structure of the peptide. Hybridization is a useful way that can combine the advantages of other peptides. In particular, a hybrid undecapeptide from cecropin A and melittin possess potent antimicrobial activity while displaying low cytotoxicity [37]. An improved version of this hybrid peptide has been developed with retro and retroenantio analogs [38,39].

In this study, a chimeric peptide, Hn-Mc (FKRLKKLISWIKRKRQQ-NH_2), was designed to reduce the cytotoxic effects and have both antimicrobial and anti-inflammatory activities. The *in vivo* antibacterial activity of the peptide was assessed in mouse model infected by drug-resistant *Pseudomonas areuginosa*.

2. Materials and methods

2.1. Materials

Phosphatidylethanolamine (PE, from *Escherichia coli*), rhodamine-PE, and phosphatidylglycerol (PG, from *E. coli*) were from Avanti Polar Lipids (Alabaster, AL). Fluorescein isothiocyanate (FITC)-labeled lipopolysaccharide (LPS), *E. coli* O111:B4 LPS and ciprofloxacin were

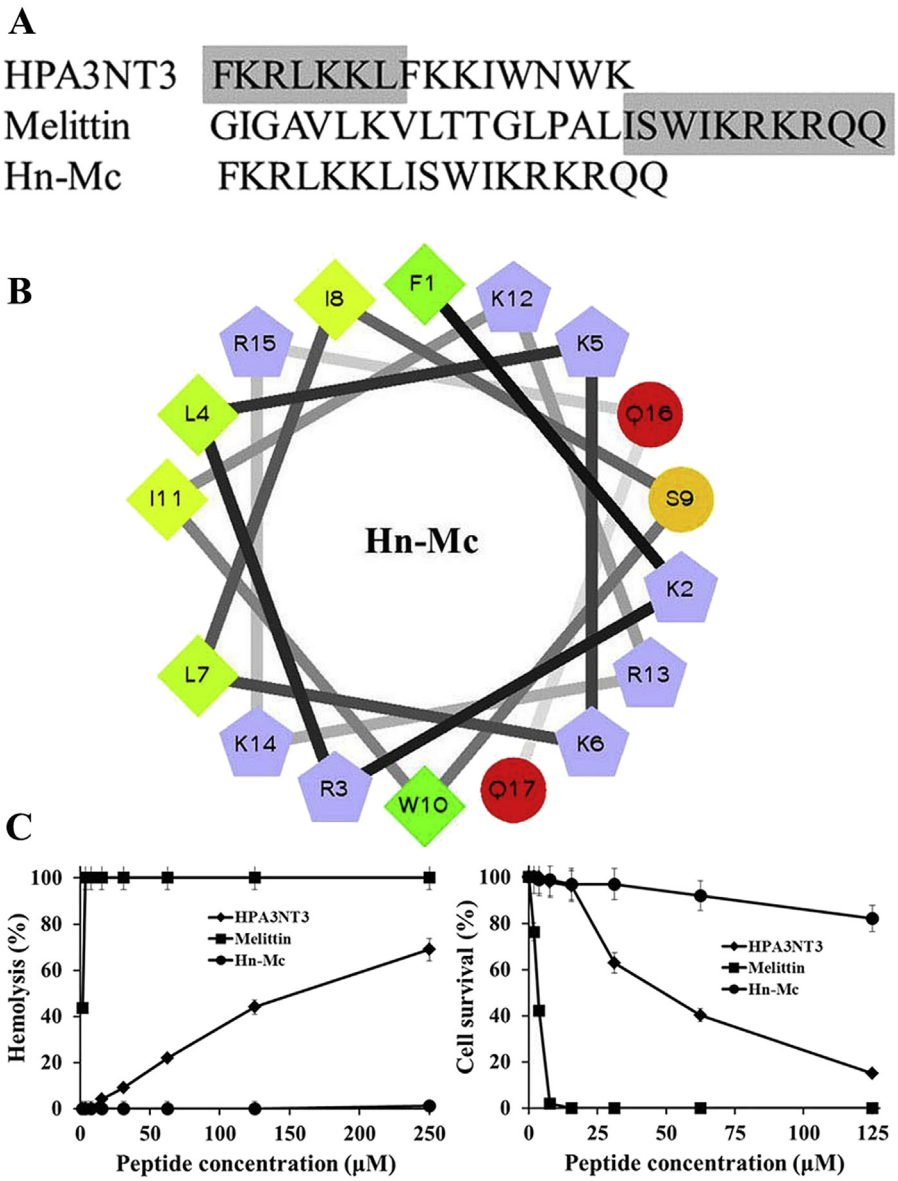

Fig. 1. Design (A), helical wheel projection (B), and cytotoxic effects (C) of the chimeric antimicrobial peptide, Hn-Mc. Hn-Mc peptide was hybridized by N-terminus of HPA3NT3 (1–7) and C-terminus of melittin (17–26) (A). The helical wheel projection was performed using online program of the Helical Wheel Projections: http://rzlab.ucr.edu/scripts/wheel/wheel.cgi (B). Synthetic peptides were evaluated for hemolysis (left panel) and cytotoxicity (right panel) in rat red blood cells and HaCaT cells, respectively (C).