

In Vitro Therapeutic Effect of a Synthetic All-D Antimicrobial Peptide against Clinically Isolated Drug-Resistant Strains

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Introduction

Conventional antibiotics have become indispensable in the modern health care system, assisting and complementing the native immune system. However, the recent appearance of antibiotic-resistant strains has necessitated a search for more potent and efficacious antibiotic agents. Over the past decade, a variety of antibacterial peptides have been identified in a wide range of species [1], serving as important components of innate immunity and the host defense systems of insects, amphibians and mammals [2-3]. Among the more potent of these antimicrobial compounds are small bioactive peptides, such as cecropin A (CA), magainin 2 (MA), melittin, HP (2-20) and plant defensin [4].

In an earlier study, we described the CA-MA-20mer hybrid, which showed greater antimicrobial activity than either CA or MA alone, yet had no hemolytic activity. Likewise, its analogue, P5-18mer, showed a similarly enhanced activity [5] and exerted a potent synergistic effect with chloramphenicol against various cell lines [6]. Therefore, in the present study we examined the antimicrobial activities of CA-MA-20mer and P5-18mer, as well as the all-D-amino acid isomer of P5-18mer (D-P5-18mer), against ten clinically isolated antibiotic-resistant bacteria under a variety of conditions, and we compared their activities to those of HP(2-20) and melittin. We also evaluated the cytotoxicity of these peptides against human cells.

Results and Discussion

We previously described the hybrid peptide CA (1-8)-MA (1-12) (CA-MA-20mer) and its analogues, which were designed to increase not only the net positive charge through Lys substitution, but also the hydrophobic helical region through Leu substitution. In particular, P5-18mer, which was created by adding a flexible region (GIG→P) and several Lys (at positions 4, 8, 14 and 15) and Leu (at positions 5, 6, 12, 13, 16, 17 and 20) substitutions, possessed broad-spectrum antimicrobial activity. In the present study, we describe the antibacterial activity of an all-D-amino acid isomer of P5-18mer (D-P5-18mer). Similar to CA-MA-20mer and P5-18mer, D-P5-18mer showed potent antibacterial activity against both Gram-positive and Gram-negative bacteria based on minimal inhibition concentrations defined by the National Committee for Clinical Laboratory Standards (NCCLS). The antimicrobial activity of D-P5-18mer was two times stronger against Gram-negative bacteria than either

CA-MA-20mer or P5-18mer (Fig. 1). When tested against ten clinically isolated antibiotic-resistant strains in the presence of 0, 150 or 300 mM NaCl, D-P5-18mer retained strong activity against all bacteria, yet showed little or no hemolytic activity or cytotoxicity against the HaCaT human keratinocyte cell line. Finally, D-P5-18mer was the only peptide tested that was resistant to trypsin degradation (Fig. 2).

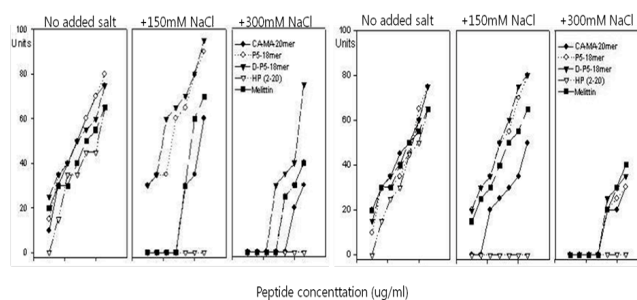


Figure 1. Effect of salinity on antimicrobial activity of the indicated peptides against *E. coli* (MDREC) and *S. typhimurium* (MDRST) in radial diffusion assays. The indicated concentrations of NaCl were added to underlay agars containing 9 mM sodium phosphate, 1mM sodium citrate buffer, 1% (wt/vol) agarose and 0.3 mg of TSB/ml. The diameters of the clear zones are given in terms of units (10 U = 1 mm). The antimicrobial activities were graphed against the log concentration of the peptides.

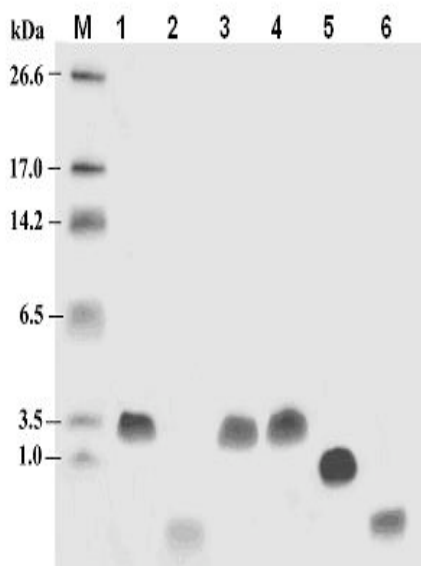


Figure 2. Stability of P5-18mer, D-P5-18mer and melittin in trypsin. Peptide samples were incubated with trypsin for 30 min and then subjected to 16% tricine gel electrophoresis. Lane M, molecular size markers (26.6, Triosephosphate isomerase; 17, Myoglobin; 14.2, α -Lactalbumin; 6.5, Aprotinin; 3.5, Insulin chain B; 1, Bradykinin). Lane 1, P5-18mer; Lane 2, P5-18mer with trypsin; Lane 3, D-P5-18mer; Lane 4, D-P5-18mer with trypsin; Lane 5, melittin; Lane 6, melittin with trypsin.

Acknowledgments

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