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Effects of the Synthetic Coprisin Analog Peptide, CopA3 in Pathogenic Microorganisms and Mammalian Cancer Cells

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A synthetic coprisin analog peptide, 9-mer dimer CopA3 (CopA3) was designed based on a defensin-like peptide, Coprisin, isolated from the bacteria-immunized dung beetle *Copris tripartitus*. Here, CopA3 was investigated for its antimicrobial activity and cancer cell growth inhibition. CopA3 showed antimicrobial activities against various pathogenic bacteria and yeast fungus with MIC values in 2~32 μM ranges, and inhibited the cell viabilities of pancreatic and hepatocellular cancer cells, except MIA-Paca2, Hep3B, and HepG2 cells, in a dose-dependent manner. The average IC_{50} values of CopA3 against pancreatic and hepatocellular cancer cells were 61.7 μM and 67.8 μM, respectively. The results indicate that CopA3 has potential in the treatments of pancreatic and hepatocellular cancers as well as microorganism infection disease.

Keywords: Coprisin analog peptide CopA3, antimicrobial peptide, defensin, antimicrobial activity

Antimicrobial peptides (AMPs) are important components found in the natural defense systems of most living organisms [9, 19]. Insect AMPs are cationic and amphipathic, with variable length, sequence, and structure, but most have relatively small (below 5 kDa) molecular masses. Moreover, they can be classified into three groups according to their structures [4, 5]. The largest group consists of peptides with intramolecular cystine disulfide bonds forming hairpin-like ß sheets or α-helical–ß sheets mixed structures. The second group contains amphipathic α-helices peptides, and the third group comprises proline-rich or glycine-rich peptides [4]. Insect defesins are members of the widely distributed family of AMPs that contain six cysteine/three disulfide bonds [4, 5] and are active against Gram-positive bacteria [3, 6] and fungi [2, 17]. Moreover, it has been reported that several insect AMPs show cytotoxic effects against broad ranges of cancer cell lines, such as mouse myeloma, melanoma, lymphomas, leukemias, breast cancer, and lung cancer [1, 11–13, 18, 21]. Thus, AMPs would be good candidates as a highly potent and effective novel therapeutic agent.

Previously, we isolated and characterized a novel insect defensin-like peptide, coprisin, from the bacteria-immunized dung beetle *Copris tripartitus* [10]. Coprisin was composed of 80 amino acids (mature protein of 43 amino acid) with a predicted molecular mass of 8.6 kDa, and was found to be 79.1% and 67.4% identical to those of defensin-like peptides of *Anomala cuprea* and *Allomyrina dichotoma,* respectively. In addition, it was reported that several 9-mer dimer analog peptides (consisted of all L amino acid sequence) derived from Leu22 to Lys30 (LHCIALRKK-NH_2) in the 43-mer coprisin peptide was synthesized to improve a more effective antimicrobial peptide. Of these synthetic 9-mer dimer peptides, CopA3 had the strongest antibacterial activities as compared with the other 9-mer analog peptides [10]. However, little is known about the effects of CopA3 toward various pathogenic microorganisms and mammalian cancer cells. Therefore, we investigated the antimicrobial activity and cancer cell growth inhibition of CopA3.

Antimicrobial Activity of CopA3 in Pathogenic Microorganism

These synthetic peptides, 9-mer dimer, CopA3 (LLCIALRKK-NH_2), and 26-mer melittin (GIGAVLKVLTTGLPALISWI KRKRQQ-NH_2), were prepared by a using solid-phase chemical synthesis (Anygen Co. at Gwangju, Korea). All strains used in this study are listed in Table 1. These strains tested were purchased from the CCARM (Culture Collection

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Table 1. Antimicrobial activities against bacteria, antibiotic-resistant bacterial strains, and fungus.

Microorganism	MIC (μM)	
	CopA3	Melittin
Gram-negative bacteria		
Escherichia coli	16	8
Klebsiella pneumoniae	16	8
Pseudomonas aeruginosa	32	32
Gram-positive bacteria		
Enterococcus faecalis	2	4
Enterococcus hirae	4	8
Staphylococcus aureus	8	4
Staphylococcus epidemidis	8	16
Antibiotic-resistant bacteria		
MDR *E. coli*	8	4
MDR *P. aeruginosa*	16	32
MR *S. aureus*	4	2
Yeast fungus		
Candida albicans	8	2

MIC values were determined in three independent experiments.

Antimicrobial Resistant Microbes, Korean) or the KACC (Korean Agricultural Culture Collection). The antimicrobial activities of CopA3 were determined by MIC (minimum inhibitory concentration) test that was performed as described previously [10]. Melittin peptide is known to have powerful antimicrobial and hemolytic activities [5, 7, 20], and was used as a positive control in the MIC test.

As expected, Cop A3 showed antibacterial activities against the pathogenic bacteria. The MIC values of Gram-positive and Gram-negative bacteria and antibiotic-resistant bacteria tested in this study were 2~8 μM, 16~32 μM, and 4~16 μM ranges, respectively (Table 1), whereas melittin also showed antibacterial activities against these bacteria with 2~32 μM ranges of MIC values (Table 1). The results confirm that CopA3 has antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* [10]. Furthermore, CopA3 had antifugal activity against human pathogenic yeast fungus, *Candida albicans*, with 8 μM of MIC value, but these effects were less strong than those of melittin (2 μM of MIC value) (Table 1). In this study, CopA3 peptide did not cause hemolysis of the mouse erythrocytes at any of the tested concentrations (data not shown). Thus, the results suggest that CopA3 has broad ranges of antimicrobial activities and potential to be considered as a novel antibiotic peptide for treating pathogenic microbial diseases without cytolysis of the red blood cells.

Cancer Cell Growth Inhibition by CopA3

Next, we determined whether CopA3 had cell growth inhibitions of mammalian pancreatic or hepatocellular cancer cells. Pancreatic (AsPc-1, Capan-1, Capan-2, MIA-PaCa-2, PANC-1, SNU-213, SNU-324, and SNU-410) and hepatocellular (Hep3B, Hep2G, SK-Hep1, SNU-182, and SNU-354) cancer cells were obtained from the KCLB (Korean Cell Line Bank). These cancer cells were maintained in RPMI-1640 or DMEM medium with 10% FBS, penicillin G (100 units/ml), and streptomycin (100 μg/ml) and incubated at 37°C in humidified 5% CO_2 and 95% air. The percentage of growth inhibition of cancer cells was analyzed using 3-(4,5-dimethylthiazo-2-yl)-5-(3-carboxymethoxyphenyl)-

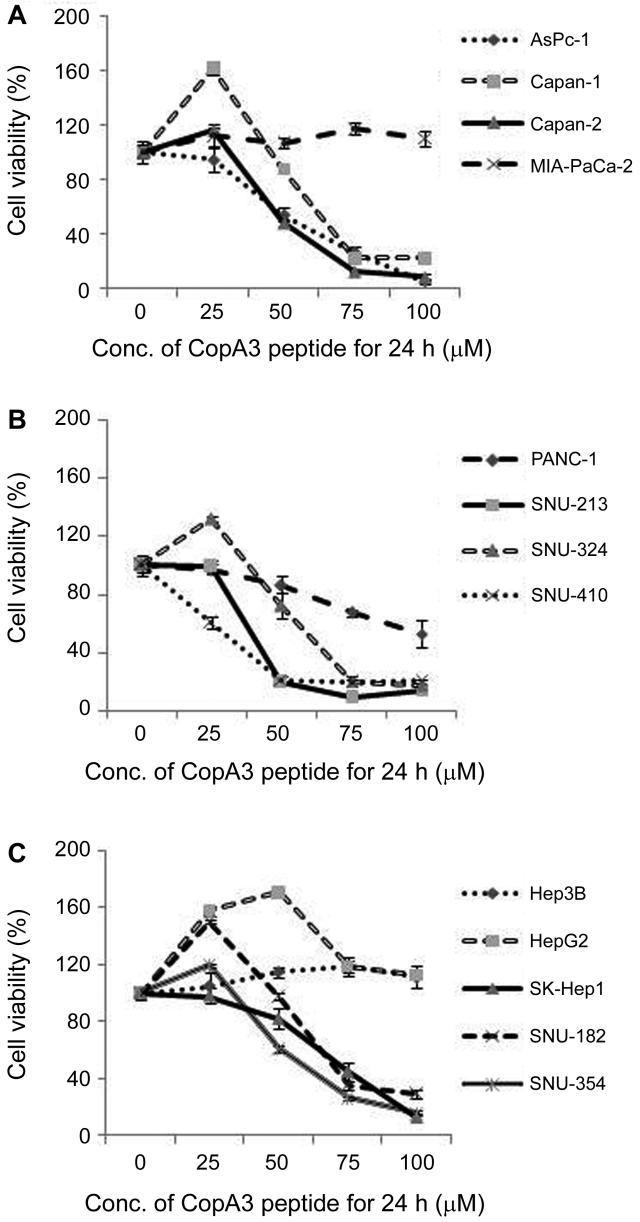

Fig. 1. The synthetic peptide, CopA3 inhibited the cell growth of human pancreatic (**A** and **B**) and hepatocellular cancer cells (**C**). These cancer cells were treated with different concentration (0, 25, 50, 75, 100 μM) of CopA3 for 24 h, and then cell viabilities were measured by the MTT assay. An error bar is used to show the standard deviation derived from three independent experiments.

2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay (Promega, USA) for the measurement of viable cells. Cancer cells were seeded in 96-well tissues culture plates containing 100 μl of cell suspension (2×10^4 cells/ml) for 24 h and were replaced with media containing various concentrations (0, 25, 50, 75, and 100 μM) of CopA3. After incubation for 24 h, the growth inhibition of cancer cells tested were measured by following the vendor's protocols for the Cell Titer 96® AQ_{ueous} One Solution cell proliferation assay (Promega, USA), and then absorbances were measured at 490 nm using a Beckman DTX8800 multi detector.

As shown in Fig. 1, CopA3 inhibited the cell growth of all cancer cells, except MIA-PaCa2, Hep3B, and HepG2, in a dose-dependent manner (Fig. 1). The average IC_{50} values of CopA3 against pancreatic and hepatocellular cancer cells were 61.7 μM (range 40.8~105.6 μM) and 67.8 μM (range 60.5~77.4 μM), respectively. Specifically, SNU-410 was the most sensitive to CopA3, with an IC_{50} value of 40.8 μM. The result indicates that CopA3 shows a selective inhibitory effect on pancreatic and hepatocellular cancer cells viability.

Recently, the synthetic peptides based on naturally occurring peptides have been reported to exhibit antimicrobial and anticancer activities [1, 8, 11, 14–16]. We found that CopA3, derived from dung beetle defensins, showed not only antimicrobial activities but also cell growth inhibitions of pancreatic and hepatocellular cancer cells. The results provide a basis for developing CopA3 as a new antibiotic peptide, although it is necessary to identify the action mechanism of CopA3 against the pathogenic microorganisms and cancer cells.

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