Anticancer activity of CopA3 dimer peptide in human gastric cancer cells

Joon Ha Lee[1,#], In-Woo Kim[1,#], Sang-Hee Kim[1], Eun-Young Yun[1], Sung-Hee Nam[1], Mi-Young Ahn[1], Dong-Chul Kang[2] & Jae Sam Hwang[1,]*

[1]Department of Agricultural Biology, National Academy of Agricultural Science, RDA, Wanju 565-851, [2]Ilsong Institute of Life Science, Hallym University, Anyang 431-060, Korea

CopA3 is a homodimeric α-helical peptide derived from coprisin which is a defensin-like antimicrobial peptide that was identified from the dung beetle, *Copris tripartitus*. CopA3 has been reported to have anticancer activity against leukemia cancer cells. In the present study, we investigated the anticancer activity of CopA3 in human gastric cancer cells. CopA3 reduced cell viability and it was cytotoxic to gastric cancer cells in the MTS and LDH release assay, respectively. CopA3 was shown to induce necrotic cell death of the gastric cancer cells by flow cytometric analysis and acridine orange/ethidium bromide staining. CopA3-induced cell death was mediated by specific interactions with phosphatidylserine, a membrane component of cancer cells. Taken together, these data indicated that CopA3 mainly caused necrosis of gastric cancer cells, probably through interactions with phosphatidylserine, which suggests the potential utility of CopA3 as a cancer therapeutic. [BMB Reports 2015; 48(6): 324-329]

INTRODUCTION

Antimicrobial peptides (AMPs) have been identified and characterized from most living organisms, including humans. AMPs have common features such as low molecular weight (less than 100 aa), net positive charge (cationic), an amphipathic structure, and they are heat-stable with low antigenicity (1). Moreover, AMPs exhibit broad-spectrum activity against various microbes together with a rapid mode of action of less than a few minutes (2). AMPs are classified into five functional groups: anti-bacteria, anti-fungi, anti-virus, anti-parasites, and anti-cancer (3).

Recently, AMPs have been featured as potential anticancer therapeutic agents. Anticancer activities of AMPs have been demonstrated against a broad range of cancer cell lines (4). AMPs are emerging as novel therapeutic alternatives that could reduce the detrimental side effects (toxicity and resistance) of conventional chemotherapeutics based on their mechanism of action and specificity. Two mechanisms of anticancer activity for AMPs have been proposed: necrosis via cell membrane damage and apoptosis via the mitochondrial pathway (5, 6). Interactions between AMPs and anionic cancer cell membrane surfaces initiate the process (7). Some important characteristics specific to cancer cells are exposure of phosphatidylserine (PS), increased number of sialic acid residues and heparan sulfate, changes in membrane fluidity and pH, and increased microvilli (7). Thus, this increase in the number of negative charges (from PS, sialic acid, and heparan sulfate) on the surface of cancer cells promotes the interaction with cationic AMPs to increase membranolytic activity during necrosis. In addition, the mitochondrial membrane is a major target of AMPs that have penetrated into the cytoplasm because of its high negative charge (i.e., cardiolipin) during apoptosis (7).

Cancer is one of the leading causes of death worldwide; lung, stomach, liver, colon, and breast cancer are the most frequently diagnosed cancers (8). Among them, gastric cancer has the fourth most incidences in the world, and gastric cancer occurs at a very high rate in East Asian countries (9). In Korea, the incidence of gastric cancer is second only to thyroid cancer; 30,092 people were diagnosed with gastric cancer in 2010, which accounted for 14.9% of total cancer cases (10). Surgical removal followed by localized radiotherapy and conventional chemotherapy are the primary regimens to treat the disease. Novel therapeutic regimens for the treatment of gastric cancer are being developed to improve efficacy as well as to reduce the side effects of conventional chemotherapeutics.

Previously, we have identified an insect defensin, named coprisin, which was isolated from the *E. coli*-challenged Dung beetle, *Copris tripartitus* (11). The synthetic peptide CopA3, an analogue derived from coprisin, was shown to decrease the cell viability of human leukemia cancer cells and induce apoptosis in AML-2, Jurkat, and U937 cancer cells via a caspase-independent pathway (12). CopA3 also demonstrated antimicro-

bial activity against various microbes (13, 14). Here, we investigated the anticancer activity of the CopA3 peptide against human gastric cancer cells. We showed that the anticancer effect of CopA3 is mainly attributed to interactions with PS on the cell surface, which initiates necrosis in the cancer cells.

RESULTS

CopA3 reduced cell viability of gastric cancer cells

We investigated the effect of the synthetic peptide CopA3 on the cell viability of a human gastric cancer cell line (SNU-484, 601, 638, 668). Cancer cells were treated with various concentrations of CopA3 (10, 25, 50, and 100 μM) for 24 h, and cell viability was measured by the MTS assay. CopA3 decreased the viability of the gastric cancer cells in a dose-dependent manner (Fig. 1A). The IC$_{50}$ of CopA3 for gastric cancer cells was about 20-50 μM. In contrast, Raw 264.7 cells were relatively resistant to CopA3 treatment, which suggests some specificity of CopA3 against the gastric cancer cells.

Effect of CopA3 on cancer cell membrane integrity

We next determined the effect of CopA3 on cancer cell mem-

brane integrity by detecting the release of LDH. The amount of LDH release increased in a dose-dependent manner in gastric cancer cells, and the percentage of cytotoxic cells appeared to reach a maximum at a peptide concentration of 100 μM (Fig. 1B). Cytotoxicity to gastric cancer cells at 50 μM CopA3 was over 60.0%, while cytotoxicity to Raw 264.7 cells was 27.0% at the same concentration of CopA3. These data are consistent with the cell viability assay results, which suggest a specific cytotoxic effect of CopA3 against the gastric cancer cells.

Specific binding of CopA3 to PS on the cancer cell membrane

To delineate the cytotoxic mechanism of CopA3 on the gastric cancer cells, we determined the effect of pre-treatment with cancer cell-specific membrane components (ganglioside, heparin, and PS) on CopA3-induced reduced viability of the cancer cells. The viability of CopA3-treated cancer cells increased with increasing PS concentration in a dose-dependent manner (Fig. 2). The addition of ganglioside had less effect on cell viability in the presence of CopA3. The effect of the cancer cell membrane molecules on the antibacterial activity of CopA3 was also examined to determine the specific binding of CopA3 on the cancer cell surface. A constant amount of CopA3 (1 μg)
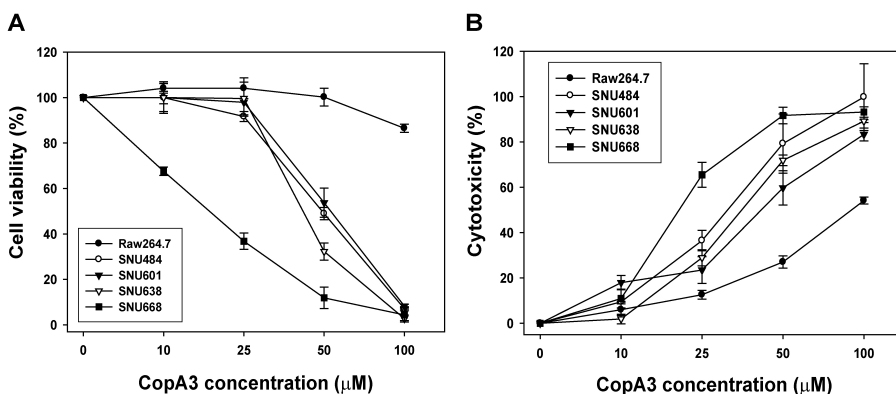

Fig. 1. Cell viability and cytotoxicity of human gastric cancer cells after CopA3 treatment. (A) Cell viability was measured by the MTS assay after 24 h incubation with indicated amount of the peptides. (B) After 24 h incubation with the peptide, LDH activity from each cell was detected using the LDH release assay. Each symbol represents the mean value from triplicate experiments and the error bars depict standard deviations.
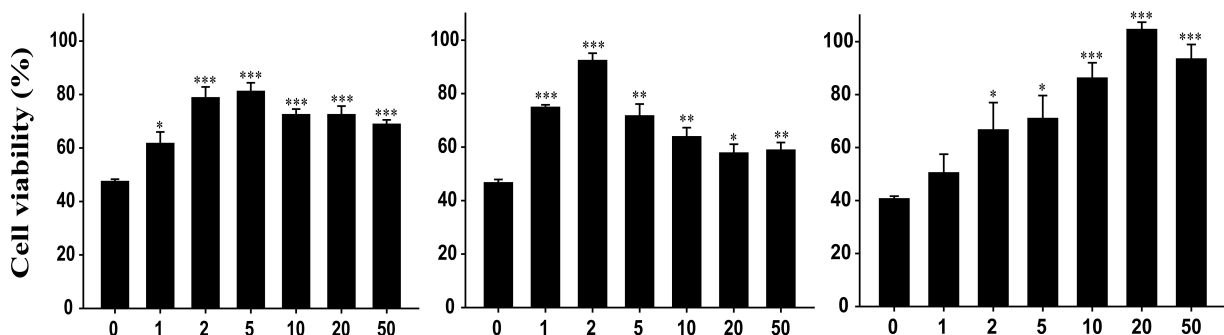

Fig. 2. Effect of anionic cancer cell-surface molecules on cell viability. Viability assay of gastric cancer cells was performed by mixing various amounts of ganglioside, heparin, or phosphatidylserine with 25 μM CopA3. Data are expressed as means ± standard deviations from three individual measurements. Statistical analyses were performed as described. ***P < 0.001, **P < 0.01, *P < 0.05, compared to the non-treated control cells.

was incubated at different concentrations of ganglioside, heparin, PS, phosphatidylcholine (PC), phosphatidylethanolamine (PE), or sphingomyelin, and then the mixture was tested for antibacterial activity in the radial diffusion assay (Fig. 3A). The antibacterial activity of CopA3 was significantly reduced as the amount of heparin and PS increased in the mixture. In contrast, the other cell membrane components did not affect the antibacterial activity of CopA3. In addition, we conducted a calcein leakage assay to elucidate the interaction of CopA3 with cell membrane molecules using artificial liposomes (Fig. 3B). Two types of calcein-entrapped liposomes consisting of PS, and sphingomyelin were prepared and the amount of calcein release was determined from the liposomes at different peptide concentrations. As a result, CopA3 induced calcein leakage from the PS liposomes. The result was consistent with the anti-*E. faecalis* activity of CopA3 in the presence of membrane components in radial diffusion assay (Fig. 3A). While polymixin B slightly induced calcein leakage from PS liposome, CopA3 well induced leakage of calcein dose-dependently. In contrast, when sphingomyelin liposome was treated with peptides, little or no calcein leakage was detected. These findings suggest that CopA3 interacts with heparin and PS, which could result in CopA3-induced cytotoxicity in cancer cells as well as in bacteria.

CopA3 induced apoptosis and necrosis in the gastric cancer cells

We assessed the nature of the cytotoxic effect of CopA3 to characterize the mechanism of reduced viability. Apoptosis of the cancer cells was examined by Annexin V/PI staining of the CopA3-treated gastric cancer cells (Fig. 4A). Annexin V binding to the CopA3-treated gastric cancer cells increased at a peptide concentration of 25 μM and 50 μM. The Annexin V-positive/PI-negative population reached maximum at 50 μM CopA3, while the Annexin V-negative/PI-positive population gradually increased as the peptide concentration increased and reached a maximum at the highest concentration of CopA3 (100 μM). These data indicate that CopA3 induced both apoptosis and necrosis in the gastric cancer cells, although necrotic cell death was dominant at higher concentrations of CopA3.

Acridine orange/ethidium bromide staining

Membrane integrity of the SNU-668 cells upon CopA3 treatment was analyzed by acridine orange/ethidium bromide staining. After CopA3 treatment for 1 h, the number of orange-colored nuclei increased in CopA3-treated cells with increasing CopA3 concentration, while the majority of control cells exhibited a green fluorescence (Fig. 4B). The cells treated with CopA3 exhibited an orange and orange-red fluorescence, indicating severe membrane disruption at high concentrations of CopA3.

DISCUSSION

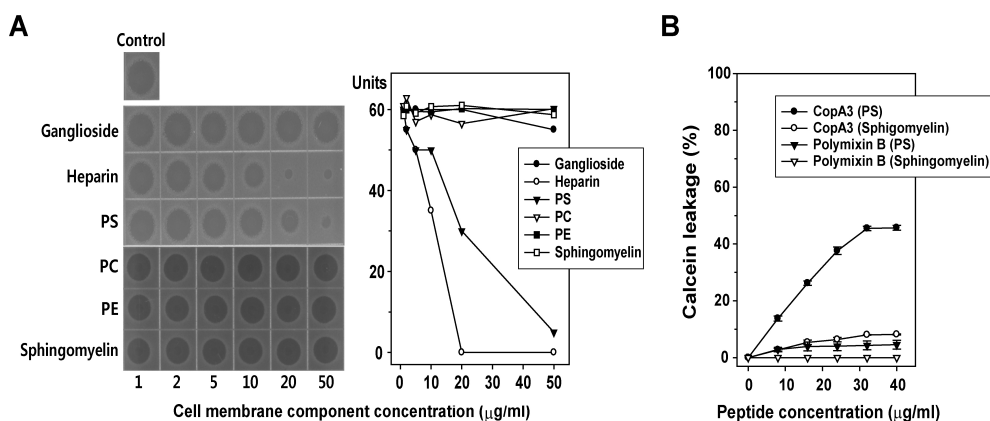We have described the anticancer activity of CopA3 against

Fig. 3. Specific binding of CopA3 to cell membrane components. (A) The radial diffusion assay was conducted by mixing various amounts of ganglioside, heparin, phosphatidylserine (PS), phosphatidylcholine (PC), phosphatidylethanolamine (PE), or sphingomyelin with 1 μg of CopA3. The mixture of peptides with the various cell membrane molecules was loaded into wells of the assay plate seeded with *E. faecalis*, which has been confirmed to be highly susceptible CopA3. The left panel shows a photo of the gel from the radial diffusion assay. The right panel shows the antibacterial activity of CopA3 in the mixture plotted against the cell membrane components concentration. The diameters of the clearing zone are expressed in units (1 mm = 10 units). The results shown are representatives of three independent experiments. (B) Percent leakage from the liposomes consisting of PS, or sphingomyelin in the calcein leakage assay. Liposomes were incubated with given amounts (8, 16, 24, 32, and 40 μg/ml) of peptides and they were measured the fluorescence intensity as described in Materials and Methods. The maximum level of fluorescence (100%) was determined by complete lysis of liposomes treated with Triton X-100. The zero level corresponded to liposomes fluorescence in the absence of peptide. Polymixin B was used as a control peptide. Each symbol represents the mean value from triplicate experiments and the error bars depict standard deviations.
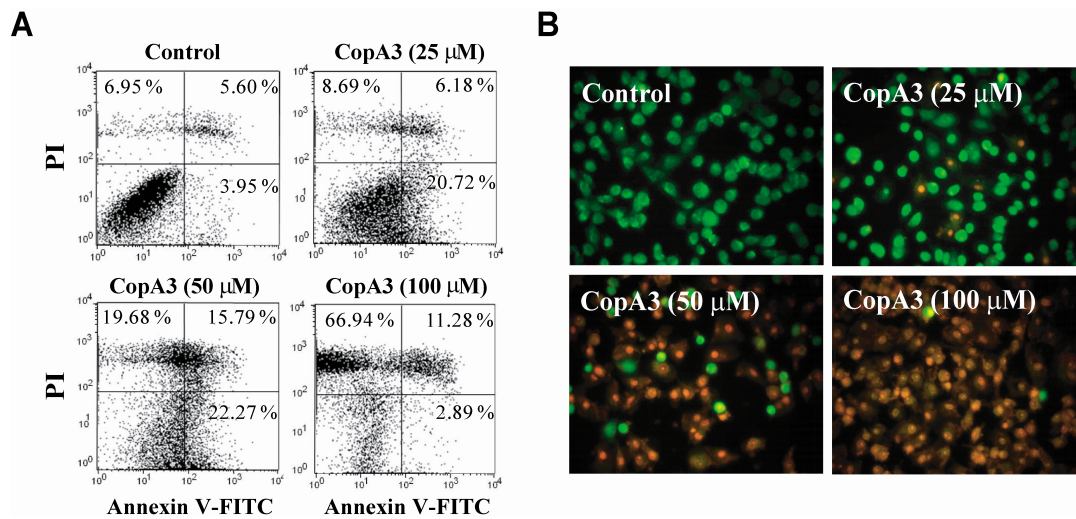
Fig. 4. CopA3 induces apoptosis and necrosis in SNU-668 human gastric cancer cells. (A) Flow cytometric analyses of the gastric cancer cells treated with various concentrations of CopA3 for 1 h. The cells were stained with annexin V-FITC and PI and subjected to flow cytometry. The distribution of the cells is presented as the percentage of total cells counted (10,000). (B) Gastric cancer cells were stained with acridine orange/ethidium bromide after incubation with CopA3. Cells were observed under a fluorescence microscope (200×). Live cells show green fluorescence, and necrotic cells show orange fluorescence.

the human gastric cancer cell lines. CopA3 selectively reduced the viability of the gastric cancer cells, while CopA3 did not exert a significant cytotoxic effect on Raw264.7 cells (Fig. 1A). Since PS and heparin inhibited CopA3-induced reduced viability of the cancer cells, the anticancer activity might be ascribed to the interaction between cationic AMPs and anionic surface molecules of cancer cells.

To investigate the anticancer mechanism of CopA3, we assessed the cytotoxicity of the peptide using the LDH release assay. CopA3 was found to disrupt the membrane integrity of the gastric cancer cells and thereby increase LDH release in a dose-dependent manner with increasing peptide concentrations (Fig. 1B). CopA3-treated Raw264.7 cells showed cytotoxic activity; however, the lytic activity was significantly lower compared to that in the gastric cancer cells. A lower percentage of FBS in the LDH release assay (1% FBS) compared to the MTS assay (10% FBS) might enhance cell death, resulting in higher LDH release at high CopA3 concentrations, which suggests that the lytic activity of CopA3 is influenced by serum levels. We examined the viability of Raw264.7 cells in serum-free conditions after CopA3 treatment and found that the viability of the cells remained > 70% at 50 μM CopA3, while the viability dropped to < 10% at 100 μM CopA3 (data not shown). These data indicate that an effective dose of CopA3 is ≤ 50 μM.

It has been reported that enantiomeric 9-mer peptide derived beetle defensins showed anticancer activity against several cancer cell lines, and the peptides selectively targeted PS on the surface of cancer cell membranes (15). In addition, diastereomeric lytic peptides also interacted with PS in PS-con-

taining model membranes (16). Therefore, we examined whether the viability of gastric cancer cells was affected by CopA3 in the presence of anionic cell surface molecules (Fig. 2). Results from these experiments strongly suggested that cell-surface PS was a target molecule for CopA3 binding. To support this hypothesis, we further analyzed the antimicrobial activity of CopA3 in the presence of cell membrane molecules by using a radial diffusion assay (Fig. 3A) and induction of calcein leakage from liposome by CopA3 treatment in calcein leakage assay (Fig. 3B). The results of the present study revealed that the binding affinity of CopA3 for PS was shown to be critical for the anticancer activity. Thus, the results from these experiments indicated that PS might be a potential target for CopA3 binding, in addition to heparin. Buforin IIb is a cell-penetrating anticancer peptide that causes apoptosis and targets cancer cells through interactions with gangliosides (17). However, CopA3 did not bind to gangliosides and retained its antibacterial activity in the presence of gangliosides. Thus, it is likely that the target molecules on the surface of cancer cell are dependent on the peptide's mechanism of action.

The mechanism of CopA3-induced cell death was further assessed by flow cytometric analysis with annexin V and PI staining (Fig. 4A). The results revealed that cell death of gastric cancer cells depend on CopA3 concentration and incubation time. Induction of apoptotic or necrotic cell death by a lactoferrin-derived peptide (Pep1) was also shown to be concentration-dependent (18). CopA3-induced necrosis was more prevalent than CopA3-induced apoptosis at a high peptide concentration. Flow cytometric analysis of Annexin V/PI binding showed CopA3-induced apoptosis predominated with a

short incubation time (1 h) (Fig. 4A) at a low CopA3 concentration, while necrotic cell death was more prevalent over an apoptosis longer incubation time (4 h) at both low and high CopA3 concentrations (data not shown). These data suggest that CopA3-induced apoptosis is transient and was only initiated at low CopA3 concentrations. In addition, we stained CopA3-treated SNU-668 cells with acridine orange/ethidium bromide to verify the annexin V/PI staining results (Fig. 4B). These results indicated that CopA3-treated cells were not undergoing apoptosis; strong staining with EtBr indicated that the cells were primarily necrotic.

In conclusion, our findings demonstrate that CopA3 displays selective anticancer activity in human gastric cancer cells. *In vitro* analysis by the LDH release assay, flow cytometric analysis, and acridine orange/ethidium bromide staining indicated that CopA3 primarily induced necrosis in the cancer cells. The anticancer and antimicrobial activities of CopA3 were reversed by incubation with heparin and PS, which suggests that these activities are dependent on an interaction between CopA3 and heparin/PS. Collectively, these results suggest the potential utility of CopA3 as a therapeutic for gastric cancer.

MATERIALS AND METHODS

Peptide
The homodimeric peptide CopA3 was synthesized using the solid-phase peptide synthesis method by Anygen Co., Ltd. (Gwangju, Korea). The peptide was dissolved in acidified distilled water (0.01% acetic acid) and stored at $-20^{\circ}C$ until use. CopA3 consists of a homodimer of two subunits that contain nine residues (LLCIALRKK-NH$_2$) each. The two monomers are linked by a single disulfide bond between the third Cys of each monomer. The total net charge of CopA3 is +6.

Cell culture
Raw 264.7, Caki, and HeLa cells were maintained in DMEM, and SNU-668 cells were maintained in RPMI-1640 medium supplemented with 10% FBS, penicillin G (100 U/ml), and streptomycin (100 μg/ml) (Invitrogen, USA). Cells were cultured at $37^{\circ}C$ in a humidified incubator with 5% CO_2.

MTS assay for cell viability
Cells plated into 96-well tissue culture plates (2×10^4 cells per well) on the previous day were treated with various concentrations (10, 25, 50, and 100 μM) of CopA3 or without CopA3. After incubation for 24 h, the viability of the cancer cells was assessed by the Cell Titer 96 AQueous One Solution Cell Proliferation Assay according to the manufacturer's protocol (Promega, USA). The optical density at 490 nm was measured with a microplate reader (Beckman DTX 8800 multi detector). To investigate the interaction of CopA3 with cell membrane components, cells were treated with ganglioside, heparin, or PS at the indicated concentration in the presence of CopA3. Then, cell viability was measured as described above.

LDH release assay
Cell membrane integrity was analyzed by measuring LDH activity. LDH activity was monitored using a Cytotoxicity Detection Kit (Roche Applied Science, Germany). In brief, cells were seeded at 1×10^4 cells per well in a 96-well culture plate in assay medium (DMEM or RPMI-1640 containing 1% FBS) and were treated with different doses of CopA3. After 24 h of incubation, 5 μl of lysis solution was added to high control samples as a positive control, and the plate was incubated for an additional 15 min. Then, 100 μl of the reaction mixture was added to each well, followed by a 15-min incubation. Finally, 50 μl stop solution was added to each well, and the absorbance was measured using a microplate reader at 490 nm. The percent cytotoxicity was calculated by the following equation: Cytotoxicity (%) = (exp. value - low control) / (high control $-$ low control) $\times$ 100

Binding of CopA3 to cancer cell-specific membrane components using the radial diffusion assay
The ability of CopA3 to bind to the surface of SNU-668 cells was examined by assessing the effect of cancer cell membrane components on the anti-*E. faecalis* activity of CopA3 in a radial diffusion assay (19). One microgram of CopA3 was incubated with different amounts of ganglioside, heparin, PS, phosphatidylcholine (PC), phosphatidylethanolamine (PE), or sphingomyelin for 10 min at $37^{\circ}C$ in 10 mM sodium phosphate buffer (pH 7.4). Then, 5-μl aliquots of each mixture were loaded into wells (3-mm diameter) that had been punched in the underlay agar containing washed mid-logarithmic *E. faecalis* (4×10^6 colony forming units). The underlay agar consisted of 9 mM sodium phosphate, 1 mM sodium citrate buffer, 1% (w/v) agarose (Sigma, A6013), and 0.3 mg of tryptic soy broth (TSB: Difco). After incubation at $37^{\circ}C$ for 3 h, a 10-ml overlay agar of 1% agarose and 6% TSB was poured onto the underlay agar. The diameters of the clearing zones were used to indicate the antibacterial activity, which were plotted against the cell membrane component concentrations.

Binding of CopA3 to cancer cell-specific membrane components using calcein leakage assay
The lipid vesicles were prepared and the calcein leakage assay was performed according to the procedure described previously (20). Calcein-entrapped vesicles were obtained by elution with 10 mM Tris buffer (pH 7.4) containing 150 mM NaCl on a Sephadex G-50 column. In the calcein leakage assay, the fluorescence intensity was measured using a fluorometer (VARIAN, USA) with excitation and emission values of 490 and 520 nm, respectively. The percent dye-release was evaluated by the following equation: leakage (%) = (F $-$ F$_0$) / (F$_{max}$ $-$ F$_0$) $\times$ 100, where F$_0$ was the fluorescence intensity of the control vesicle, and F and Fmax were the fluorescence intensities achieved by peptides and 1% (v/v) Triton X-100, respectively.

Annexin V/Propidium Iodide (PI) staining

SNU-668 cells were plated into 6-well tissue culture plates (1 × 10^6 cells per well) and treated with various concentrations of CopA3 (25, 50, and 100 μM) or without CopA3. After incubation for 1 h, cells were harvested and washed twice with cold PBS and resuspended in 1× Binding buffer (0.01 M Hepes/NaOH (pH 7.4), 0.14 M NaCl, 2.5 mM CaCl$_2$). Cells (1 × 10^5 cells) were then transferred to a 1.5-ml tube containing 100 μl of binding buffer, and 5 μl of FITC-conjugated Annexin V and propidium iodide (PI) was added. The cells were vortexed gently and incubated for 15 min at room temperature in the dark. After incubation, 400 μl of 1× binding buffer was added to each tube. Stained cells were measured by flow cytometry with a BD FACSCalibur cytometer (BD Biosciences, USA) and CellQuest software (BD Biosciences, USA) was used for analysis of the results.

Acridine orange/ethidium bromide staining

Cells were seeded in 6-well tissue culture plates (1 × 10^6 cells per well), treated with CopA3 (25, 50, and 100 μM) or without CopA3 for 1 h, and washed with PBS. Cells were then stained with acridine orange (3 μg/ml) and ethidium bromide (10 μg/ml) and observed immediately under a fluorescence microscope (Carl Zeiss, Germany).

Statistical analysis

Data are presented as an average with standard deviation (mean ± SD) of at least three independent experiments. Differences among groups were evaluated by Duncan post-hoc ANOVA analysis and considered statistically significant at $P < 0.05$.

ACKNOWLEDGEMENTS

REFERENCES

1. Powers JP and Hancock RE (2003) The relationship between peptide structure and antibacterial activity. Peptides 24, 1681-1691
2. Jenssen H, Hamill P and Hancock RE (2006) Peptide antimicrobial agents. Clin Microbiol Rev 19, 491-511
3. Li Y, Xiang Q, Zhang Q, Huang Y and Su Z (2012) Overview on the recent study of antimicrobial peptides: origins, functions, relative mechanisms and application. Peptides 37, 207-215
4. Hoskin DW and Ramamoorthy A (2008) Studies on anticancer activities of antimicrobial peptides. Biochim Biophys Acta 1778, 357-375
5. Papo N and Shai Y (2005) Host defense peptides as new weapons in cancer treatment. Cell Mol Life Sci 62, 784-790
6. Schweizer F (2009) Cationic amphiphilic peptides with cancer-selective toxicity. Eur J Pharmacol 625, 190-194
7. Riedl S, Zweytick D and Lohner K (2011) Membrane-active host defense peptides-Challenges and perspectives for the development of novel anticancer drugs. Chem Phys Lipids 164, 766-781
8. Parkin DM, Bray F, Ferlay J and Pisani P (2005) Global cancer statistics, 2002. CA Cancer J Clin 55, 74-108
9. Roukos DH (2000) Current status and future perspectives in gastric cancer management. Cancer Treat Rev 26, 243-255
10. Jung KW, Won YJ, Kong HJ, Oh CM, Seo HG and Lee JS (2013) Cancer statistics in Korea: incidence, mortality, survival and prevalence in 2010. Cancer Res Treat 45, 1-14
11. Hwang JS, Lee J, Kim YJ et al (2009) Isolation and characterization of a defensin-like Peptide (coprisin) from the dung beetle, Copris tripartitus. Int J Pept doi:10.1155/2009/136284
12. Kang BR, Kim H, Nam SH et al (2012) CopA3 peptide from Copris tripartitus induces apoptosis in human leukemia cells via a caspase-independent pathway. BMB Rep 45, 85-90
13. Kang JK, Hwang JS, Nam HJ et al (2011) The insect peptide coprisin prevents Clostridium difficile-mediated acute inflammation and mucosal damage through selective antimicrobial activity. Antimicrob Agents Chemother 55, 4850-4857
14. Kim IW, Kim SJ, Kwon YN et al (2012) Effects of the synthetic coprisin analog peptide, CopA3 in pathogenic microorganisms and mammalian cancer cells. J Microbiol Biotechnol 22, 156-158
15. Iwasaki T, Ishibashi J, Tanaka H et al (2009) Selective cancer cell cytotoxicity of enantiomeric 9-mer peptides derived from beetle defensins depends on negatively charged phosphatidylserine on the cell surface. Peptides 30, 660-668
16. Papo N and Shai Y (2003) New lytic peptides based on the D,L-amphipathic helix motif preferentially kill tumor cells compared to normal cells. Biochemistry 42, 9346-9354
17. Lee HS, Park CB, Kim JM et al (2008) Mechanism of anticancer activity of buforin IIb, a histone H2A-derived peptide. Cancer Lett 271, 47-55
18. Onishi J, Roy MK, Juneja LR, Watanabe Y and Tamai Y (2008) A lactoferrin-derived peptide with cationic residues concentrated in a region of its helical structure induces necrotic cell death in a leukemic cell line (HL-60). J Pept Sci 14, 1032-1038
19. Steinberg DA and Lehrer RI (1997) Designer assays for antimicrobial peptides. Methods Mol Biol 78, 169-186
20. Chongsiriwatana NP and Barron AE (2010) Comparing bacterial membrane interactions of antimicrobial peptides and their mimics. Methods Mol Biol 618, 171-182