CopA3 peptide from *Copris tripartitus* induces apoptosis in human leukemia cells via a caspase-independent pathway

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Our previous study demonstrated that CopA3, a disulfide dimer of the coprisin peptide analogue (LLCIALRKK), has antibacterial activity. In this study, we assessed whether CopA3 caused cellular toxicity in various mammalian cell lines. CopA3 selectively caused a marked decrease in cell viability in Jurkat T, U937, and AML-2 cells (human leukemia cells), but was not cytotoxic to Caki or Hela cells. Fragmentation of DNA, a marker of apoptosis, was also confirmed in the leukemia cell lines, but not in the other cells. CopA3-induced apoptosis in leukemia cells was mediated by apoptosis inducing factor (AIF), indicating induction of a caspase-independent signaling pathway. [BMB reports 2012; 45(2): 85-90]

INTRODUCTION

Recently, we isolated the peptide coprisin consisting of 43 amino acids from *Copris tripartitus*, a Korean dung beetle, and found that it had antibacterial activity. Through the subsequent observation that the α-helix region was the active domain, the 9 amino acid (AA) peptide (LLCIALRKK) corresponding to this domain was synthesized. We also found that CopA3, a disulfide dimer form of the nine AA peptide, had higher antimicrobial activity than that of the native peptide (1). The amino acid sequence of Coprisin is very similar to the sequence of defensin peptides. Defensin-like peptides are known to disrupt the bacterial membrane or to suppress cell-cycle signaling (2-5).

In the current study, we found that CopA3 selectively caused apoptosis in leukemia cells but not in other cancer cells. This activity was mediated by activation of AIF (apoptosis inducing factor), which is a regulator of a caspase-independent programmed cell death pathway (6). These results suggest a possible therapeutic use of CopA3 for inducing apoptosis of leukemia cells, in addition to its application as an antibiotic.

RESULTS

CopA3 causes a marked decrease in cell viability in leukemia cell lines

Because CopA3 is known to have an antibiotic effect on bacteria (1), we assessed whether CopA3 affected the viability of mammalian cells. Various human cell lines including Caki (kidney cell carcinoma), HeLa (uterine cervix adenocarcinoma), AML-2 (acute myeloblastic leukemia), Jurkat (acute T cell leukemia), and U937 (histiocytic lymphoma) cells were exposed to CopA3 for 12 h, and cell viability was measured by the MTS assay. As shown in Fig. 1A, CopA3 decreased cell viability in AML, Jurkat, and U937 cells in a dose-dependent manner, whereas CopA3 had no effect on the viability of Caki and HeLa cells. A marked reduction in cell viability was confirmed at 12 h, and this was retained for 48 h, except in AML-2 cells, which recovered after 24 h of exposure (Fig. 1B). Moreover, exposure of Jurkat cells to CopA3 for 12 h caused a greater decrease in cell viability than that of the native peptide, Coprisin (Fig. 1C). Microscopic image analysis revealed that CopA3 caused cell shrinkage with a condensed nucleus and a rough plasma membrane, which are indicative of apoptosis (Fig. 1D) (7-9).

Leukemia cell specific apoptosis induced by CopA3

Next, we investigated whether CopA3 induced apoptosis in human leukemia cells. To do this, cells were treated with CopA3 for 24 h, and DNA fragmentation in the nucleus was evaluated using TUNEL staining. As shown in Fig. 2A, CopA3 significantly induced DNA fragmentation (white spots, TUNEL) in AML, Jurkat, and U937 cells, but not in non-leukemia HeLa cells. Our previous study demonstrated that CopA3, a disulfide dimer of the coprisin peptide analogue (LLCIALRKK), has antibacterial activity. In this study, we assessed whether CopA3 caused cellular toxicity in various mammalian cell lines. CopA3 selectively caused a marked decrease in cell viability in Jurkat T, U937, and AML-2 cells (human leukemia cells), but was not cytotoxic to Caki or Hela cells. Fragmentation of DNA, a marker of apoptosis, was also confirmed in the leukemia cell lines, but not in the other cells. CopA3-induced apoptosis in leukemia cells was mediated by apoptosis inducing factor (AIF), indicating induction of a caspase-independent signaling pathway. [BMB reports 2012; 45(2): 85-90]

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Apoptosis induced by CopA3 in human leukemia cells
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Fig. 1. CopA3 selectively decreases cell viability in human leukemia cell lines. (A) Leukemia cells including AML-2, Jurkat, and U937, and non-leukemia cells including Caki and HeLa, were treated with CopA3 at different concentrations. After treatment for 12 h, cell viability was measured by the MTS assay. The bars represent the mean ± SEM of 3 experiments performed in triplicate. (B) Time dependence of CopA3 on cell viability reduction. (C) Jurkat cells were treated with native peptide coprisin (native) or CopA3 for 12 h. (D) Light microscopic images after incubation of CopA3 for 24 h (×200).

CopA3-induced leukemia cell apoptosis is not associated with cell membrane disruption
Because defensin peptides have been known to cause cell membrane disruption and permeabilization (11-14), we assessed whether CopA3 caused membrane disruption in mammalian cells. To do this, cells were exposed to CopA3 for 24 h, and the level of lactic dehydrogenase (LDH) in the conditioned medium was measured by incubation with MTS (15). For maximum release of LDH, cells were treated with 2% Triton X-100 for 30 min. As shown in Fig. 3A, Triton X-100 treatment significantly increased the release of LDH in all leukemia cells, whereas there was no increase in LDH levels in cells exposed to CopA3, suggesting that CopA3 does not cause membrane disruption. To exclude the possibility that CopA3 directly inhibits LDH activity regardless of permeabilizing the cell membrane, cells were exposed to Triton X-100 for 30 min and then further incubated with CopA3. After incubation for 12 h, the activity of LDH in the conditioned medium was measured. CopA3 did not affect the activity of LDH released from cells treated with Triton X-100 (Fig. 3B).

CopA3 induces apoptosis in leukemia cells via a caspase-independent pathway
Because various death signals have been shown to increase secretion of TNF-α and IL-1β during programmed cell death (16-18), the concentrations of TNF-α and IL-1β in the medium of CopA3-treated AML-2 cells were measured. As shown in Fig. 4A, CopA3 increased TNF-α release (Fig. 4A) and mRNA transcription of TNF-α (Fig. 4B) in AML-2 cells, whereas CopA3 did not affect IL-1β expression (Fig. 4B). Jurkat and U937 cells treated with CopA3 also revealed a marked increase of TNF-α levels (data not shown). In non-leukemia Caki and HeLa cells, CopA3 had no effect on the mRNA tran-