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Apoptotic Signaling Cascade of 5-aminolaevulinic Acid-based Photodynamic Therapy in Human Promyelocytic Leukemia HL-60 Cells

Tomokazu Nagao[1]*, Kazuki Matsuzaki[2], Miho Takahashi[2] and Haruyuki Minamitani[2]

[1] Institute of Biomedical Engineering, Graduate school of Science and Technology, Keio University, Yokohama 223-8522, Japan

[2] Department of Applied Physics and Physico-Informatics, Graduate School of Science and Technology, Keio University, Yokohama 223-8522, Japan

In this study, we investigated apoptotic cell death induced by photodynamic therapy using 5-aminolaevulinic acid (ALA-PDT) in human promyelocytic leukemia cells (HL-60). ALA-PDT induced apoptosis in HL-60 cells as confirmed by DNA agarose gel electrophoresis and nuclear staining with Hoechst 33342. The apoptotic cell death was inhibited by addition of broad-spectrum caspase inhibitor Z-Asp-CH_2-DCB, indicating that the apoptotic cell death was induced in a caspase-dependent manner. Actually, western blotting analysis revealed that caspase-3 was processed as early as 1.5 h after ALA-PDT. Cytoplasmic cytochrome c released from mitochondria was detected by western blotting. However, inhibitor of caspase-9, a cysteine protease located in the downstream of cytochrome c release, was not able to reduce the apoptotic cell death. Therefore, the mitochondrial apoptotic pathway was not involved in the ALA-PDT-induced apoptosis. On the other hand, it was found that ALA-PDT-induced apoptosis was clearly inhibited by pretreatment of caspase-8 inhibitor. These data suggest that caspase-8-mediated apoptotic pathway is important in ALA-PDT-induced cell death.

Key words : photodynamic therapy, cytotoxic effect, apoptosis, caspase, ALA

INTRODUCTION

While the biochemical mechanism of the enhanced protoporphyrin IX synthesis in the cancer cells following 5-aminolaevulinic acid (ALA) induction is well understood, little is known about the mechanism of cytotoxicity of photodynamic therapy using ALA (ALA-PDT). The involvement of apoptosis has been implicated as an early event of cell death induced by PDT [1,2]. Caspases are crucial components of most apoptotic pathway. It was found that alterations in mitochondrial functions such as decrease in the mitochondrial membrane potential and the release of cytochrome c play an important role in the induction of apoptosis. In this study, we investigated detailed signal transduction of ALA-PDT-induced apoptosis in human promyelocytic leukemia cells (HL-60).

*To whom correspondence should be addressed.

E-mail : nagao@mnt.appi.keio.ac.jp

MATERIALS AND METHODS

Cell culture and ALA-PDT treatment. All experiments utilized HL-60 cells, which were routinely grown in 75 cm^2 tissue culture flasks with RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). HL-60 cells were loaded with ALA by incubating a cell suspension (about 2×10^5 cells/ml) with medium containing 10% FBS and 1.0 mM ALA in the dark at 37℃ for 4 h. The cells were washed with fresh medium and illuminated using a 10 cm × 10 cm LED array delivering red light (λ =626 nm, light intensity = 5 mW/cm^2). The cells were further incubated in an incubator before using in each assay.

Apoptosis assay. Apoptosis was detected by agarose gel electrophoresis. Rates of apoptosis were quantified using fluorescence images of HL-60 cells stained with Hoechst 33342. The number of apoptotic cells and total cells were counted and the retios of apoptotic cell to total cell were calculated. Using this parameter, inhibitory effects of broad-spectrum caspase inhibitor Z-Asp-CH_2-DCB, caspase-9 inhibitor Z-LEHD-fmk and caspase-8 inhibitor Z-IETD-fmk on apoptosis were determined. Immunoblot analysis was performed on caspase-3, caspase-8 and cytochrome c.

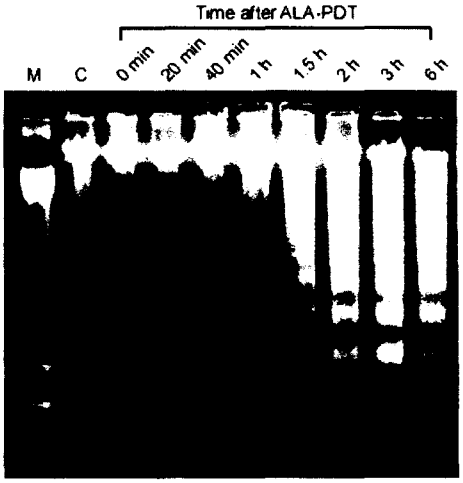

Figure 1. DNA fragmentation of HL-60 cells induced by ALA-PDT.

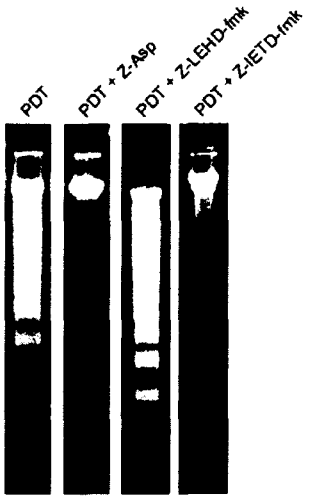

Figure 2. Effects of caspase inhibitors on ALA-PDT-induced DNA fragmentation.

RESULTS AND DISCUSSION

After ALA-PDT, DNA fragmentation was assessed by agarose gel electrophoresis. Apoptotic cell death was confirmed by oligonucleosomal DNA fragmentation (Fig. 1). The DNA ladder pattern was observed as early as 1.5 h after ALA-PDT.

Next, we determined whether caspase family proteases are involved in the apoptotic response of HL-60. HL-60 cells were treated with ALA-PDT in the presence of caspase inhibitors, Z-Asp-CH_2-DCB, Z-LEHD-fmk and Z-IETD-fmk. The DNA ladder pattern was completely diminished by addition of Z-Asp-CH_2-DCB or Z-IETD-fmk, while Z-LEHD-fmk failed to suppress the DNA fragmentation (Fig.2). Similarly, percentage of apoptotic

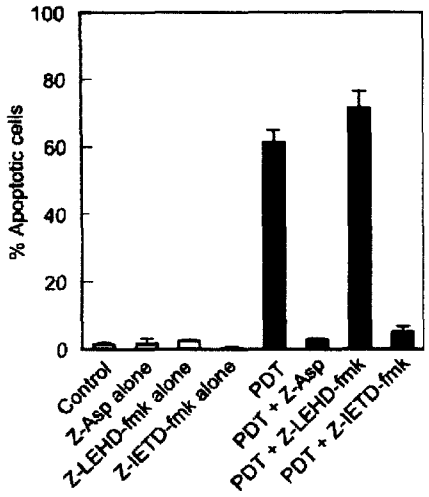

Figure 3. Effects of caspase inhibitors on percentage of apoptotic HL-60 cells.

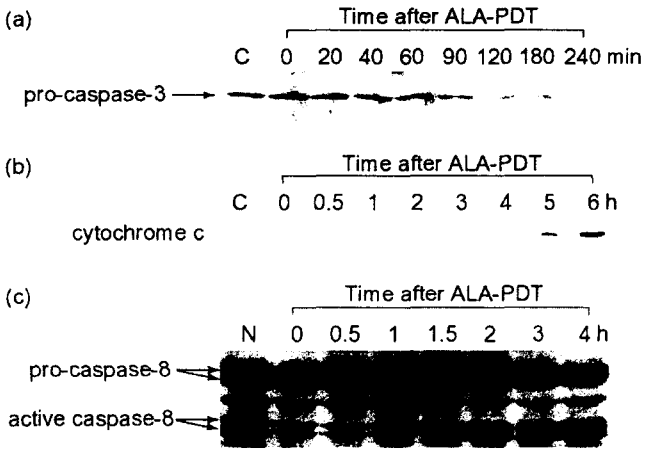

Figure 4. Cleavage of caspase-3, 8 and cytosolic translocation of cytochrome c in HL-60 cells treated with ALA-PDT.

cells was reduced by pretreatment of Z-Asp-CH_2-DCB or Z-IETD-fmk (Fig. 3). On the other hand, Z-LEHD-fmk was not able to reduce the percentage of apoptotic cells.

Among the 14 identified caspases, caspase-3 is believed to be the primary executioner of apoptosis, and is activated by many death signals. Therefore, we assessed the status of caspase-3 during ALA-PDT-induced apotosis. Caspase-3 was cleaved as early as 90 min after ALA-PDT (Fig. 4 (a)). Induction of mitochondrial permeability transition and cytosolic translocation of cytochrome c are considered essential components of the apoptotic pathway. Hence, we next analyzed the cytosolic release of cytochrome c from mitochondria. Cytochrome c was released as early as 3 h after ALA-PDT (Fig.4 (b)). The cytosolic release of cytochrome c was too late as compared to the initiation of DNA fragmentation and caspase-3 processing (Fig. 1, 4 (a)). These results indicate that mitochondrial apoptotic signaling is not dominant in this apoptotic process.

Another initiator of apoptosis is the binding of ligand to death receptors such as Fas, TNFR1 and TRAIL-R1/2, which processes caspase-8 cleavage and subsequently activates other caspases. Actually, cleavage of caspase-8 was confirmed by western blotting, and caspase-8 was processed as early as 1 h after ALA-PDT (Fig. 4 (c)). Therefore, it is concluded that caspase-8-mediated apoptotic pathway plays an important role in ALA-PDT-induced cell death of HL-60 cells.

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