

Apoptosis Induction by Aluminum Phthalocyanine Tetrasulfonate-Based Photodynamic Therapy in HL-60 Cells

水溶性アルミニウムフタロシアニンの音響化学的活性化による
アポトーシス誘導

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1. Introduction

Ultrasound has an appropriate tissue attenuation coefficient for penetrating intervening tissues to reach non-superficial objects while maintaining the ability to focus energy into small volumes. This is a unique advantage when compared to electromagnetic modalities such as laser beams in the application to non-invasive treatment of deep-seated tumors.[1] Recently, it has been reported that sonodynamic therapy is a new and promising strategy for cancer treatment. It is based on the generation of the reactive oxygen species under irradiation by ultrasound in the presence of sonosensitizers that have been selectively accumulated in the target malignant tissue. [2-3] However, the sonosensitive compounds such as hematoporphyrin and Porfimer Sodium were reported to have several clinical side effects, such as severe photodermatitis, because of its long retention time in the skin.

Recently, certain sulfonated phthalocyanines have been developed as the second-generation photosensitizers for photodynamic therapy. They are eliminated more quickly from the body and cause less significant side effects than Porfimer Sodium. Among these phthalocyanines, aluminum phthalocyanine tetrasulfonate, showed the longest lifetime in the reactive triplet state when activated by photons, which can be a great advantage in the efficient generation of reactive oxygen species.[4]

In recent years, ultrasonic exposure has been shown to trigger apoptosis in malignant cells. But only a few report on the effects of sonochemically active agents on ultrasonically induced apoptosis have been published. Therefore, in this study, we examined whether the sonochemically activated phthalocyanine AIPcTS can enhance ultrasonically induced apoptosis in human promyelocytic leukemia (HL-60) cells and the reactive oxygen species are involved in the process.

2. Materials and Methods

Ultrasound Exposure Apparatus

HL-60 cells were suspended at a concentration of 4×10^6 cells/ml in RPMI 1640 (serum free), and transferred into a cylindrical polystyrene tissue culture tube rotated at 60 rpm for exposure to ultrasound. HL-60 cells were exposed to 2 MHz ultrasound for up to 3 min in the presence and absence of AIPcTS.

Evaluation of Apoptosis

Apoptosis is characterized by morphological changes. HL-60 cells were examined using a phase contrast inverted microscope at 400× magnification. The fraction of apoptotic cells was determined by counting the number of trypan blue-unstained cells showing morphological changes on a hemocytometer glass plate.

Analysis of DNA Fragmentation.

The lysed cell samples were treated with DNase-free RNase, followed by proteinase K and separated by electrophoresis on 1.5% agarose gels containing ethidium bromide.

Measurement of Caspase-3 Activity

Caspase-3 activity was assayed by using the specific fluorogenic substrate Ac-DEVD-AFC. The formation of 7-amino-4-trifluoromethylcoumarin produced from Ac-DEVD-AFC by caspase-3 was measured using a fluorescence spectrophotometer.

Measurements of Reactive Oxygen Species.

The combination of ESR and spin trapping agents were used to measure reactive oxygen species. Ultrasonically induced nitroxide production in the presence and absence of AIPcTS was measured by ESR spectroscopy in an aqueous solution of 50 mM 2,2,6,6-tetramethyl-4-piperidol-N-oxyl in the presence and absence of oxygen scavengers.

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3. Results and Discussion

The number of apoptotic cells after combined treatment of 80 μM AIPcTS and ultrasound was significantly higher than those of other treatments of ultrasound alone and AIPcTS alone (Fig.1). Also, DNA ladder formation and caspase-3 activation (Fig.2) were observed in cells treated with ultrasound and AIPcTS but not in cells treated with ultrasound or AIPcTS alone. In addition, the combination of AIPcTS and ultrasound enhanced nitroxide generation in the same acoustical arrangement. Sonodynamically induced apoptosis, caspase-3 activation, and nitroxide generation were significantly suppressed by histidine (Fig.3).

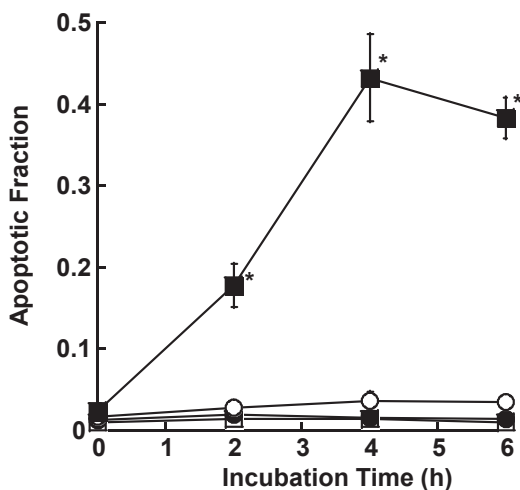


Fig.1 Fraction of apoptotic HL-60 cells after a 3-min exposure to ultrasound in the presence and absence of AIPcTS, □, no treated; ●, 80 μM AIPcTS alone; ○, ultrasound alone; ■, ultrasound + 80 μM AIPcTS. Data are presented as the mean \pm SD of four experiments. *, $p < 0.05$.

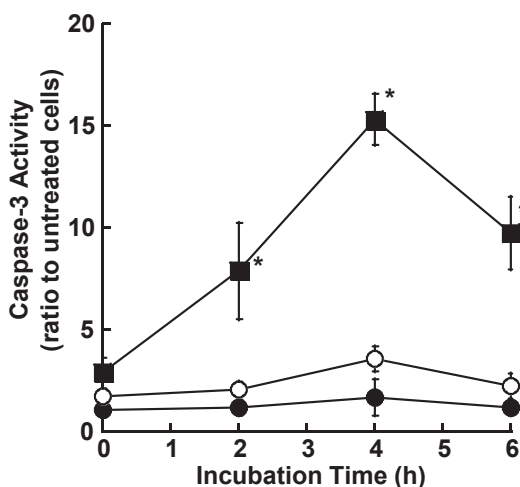


Fig.2 Caspase-3 activities in HL-60 cells after a 3-min exposure to ultrasound in the presence and absence of AIPcTS, ○, ultrasound alone; ●, 80 μM AIPcTS alone; ■, ultrasound + 80 μM AIPcTS. Data are presented as the mean \pm SD of four experiments. *, $p < 0.05$.

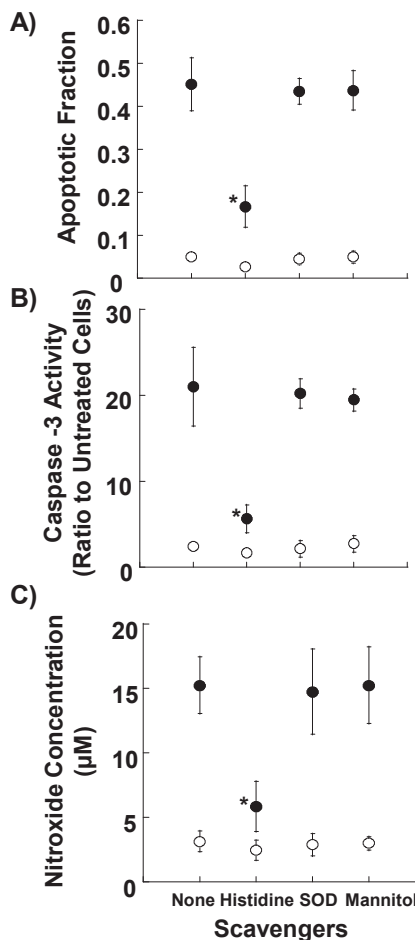


Fig.3 Effect of reactive oxygen scavengers on sonodynamically induced apoptosis (A), caspase-3 activation (B) and nitroxide generation (C), ○, ultrasound alone; ●, ultrasound + 80 μM AIPcTS. Data are presented as the mean \pm SD from four experiments. *, $p < 0.05$.

Sonodynamically induced apoptosis in HL-60 cells was demonstrated, as evidenced by morphological changes, DNA ladder formation, and caspase-3 activation. The significant reduction by histidine in the number of sonodynamically induced apoptotic cells, caspase-3 activation, and nitroxide generation suggests that ultrasonically generated reactive oxygen species such as singlet oxygen are an important mediator of sonodynamically induced apoptosis. Furthermore, these results suggest that the induction of apoptosis by sonodynamic treatment using AIPcTS might be a useful therapeutic strategy for the treatment of cancer.

4. References

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