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5-Aminolaevulinic acid enhances ultrasound-induced mitochondrial damage in K562 cells

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ABSTRACT

Background: Ultrasound therapy is a new modality in the control of malignant cancers. The aim of the present study was to investigate the effect of 5-aminolaevulinic acid on the ultrasonic killing action in the cancer cells.

Materials/methods: The K562 cells as a cancer cell model were subjected to investigate the effect of 5-aminolaevulinic acid (5-ALA) on the ultrasonic killing action, in which the 5-ALA concentration was 2 mM and the ultrasound exposure was 15 s at the intensity of 0.46 W/cm^2 and the frequency of 1.7 MHz. Cytotoxicity was investigated 24 h after ultrasound exposure using the trypan blue exclusion test. Ultrastructural cell morphology and mitochondrial changes were observed using transmission electron microscopy (TEM). Mitochondrial membrane potential ($\Delta\Psi m$) was evaluated using Rhodamine 123 assay.

Results: The death rates of the K562 cells in the controls including sham radiation and 5-ALA treatment alone were 1.81 ± 0.13%, 1.27 ± 0.20%, respectively. Those in ultrasound radiation alone and 5-ALA-ultrasound treatment were 12.61 ± 2.63%, 46.87 ± 4.09%, respectively. There were significant differences between 5-ALA-ultrasound treatment, ultrasound radiation alone and the controls ($P < 0.05$). TEM showed that the mitochondria expanding and some vacuoles were found in the ultrasound-treated cells. After the treatment of ultrasound and 5-ALA together some cells presented typical characteristics of apoptotic cells, such as nuclear condensation and crescent formation. Mitochondria of the cells were damaged more seriously than those treated by ultrasound alone, there were obvious swollen mitochondria and mitochondria in which cristae were almost perfectly disappeared, and more vacuolar mitochondria were founded. Mitochondrial membrane potential ($\Delta\Psi m$) was more significantly collapsed when the K562 cells were exposed to 2 mM 5-ALA for 4 h and then 0.46 W/cm^2 irradiation of ultrasound than ultrasound radiation alone.

Conclusion: 5-ALA pretreatment significantly enhanced the cytotoxicity of ultrasound radiation in K562 cells. The damage of mitochondria structure and function might be an important cause of cell death in K562 cells induced by the treatment of ultrasound radiation and 5-ALA together.

1. Introduction

Malignant tumor is still one of refractory diseases threatening the lives and health of human being worldwide. The current therapeutic modalities commonly have limited success with serious side-effect. Developing novel efficient method to treat malignant tumor is being pursued.

Recent experimental studies have revealed that sonodynamic therapy (SDT) is a new and effective approach to treat malignant tumor. SDT deactivates the malignant cells depending on cytotoxic reactive oxygen species (ROS) produced by ultrasound-activating sensitizer (sonosensitizer) [1–3]. Recent studies from Wang, et al. showed that the treatment with ultrasound and protoporphyrin IX (PPIX) together increased the cell damage rate of S180 cells to 50.91%, while the treatment with ultrasound alone gave a cell damage rate of 24.24%, and PPIX alone kept this rate unchanged, which demonstrated that PPIX was a potential sonosensitizer [4].

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5-Aminolaevulinic acid (5-ALA), a precursor of exogenous sensitizer porphyrins, could be changed into tissue PPIX by biosynthesis [5,6]. The present study aims to investigate whether 5-ALA could enhance the ultrasonic killing action *in vitro*.

2. Materials and methods

2.1. Sensitizer

5-Aminolaevulinic acid (5-ALA) was supplied by Sigma–Aldrich. A stock solution was made in Roswell Park Memorials Institute (RPMI)-1640 medium at a concentration of 100 mM and kept in dark at −20 °C.

2.2. Cell culture

Chronic myelogenous leukemia cell line K562 cells as cancer model cells were provided by Laboratory Center, Southwest Hospital, Third Military Medical University. Cells were grown in suspension cultures employing RPMI-1640 medium supplemented with 10% fetal calf serum (FCS, Gibco), 50 μg/ml penicillin, 50 μg/ml streptomycin and 10 μg/ml neomycin. Cells were incubated at 37 °C in a humidified CO_2 (5%) incubator.

2.3. Ultrasound treatment

The K562 cells were exposed to ultrasound at 4 h after the incubation of 5-ALA (2 mM). For ultrasound exposure, the K562 cells (1×10^6 cells/ml) in a test tube which the bottom was replaced by a latex film from the condom were put on the platform containing a ultrasound transducer as the same as described in our pervious paper [7,8]. The intensity was set at 0.46 W/cm^2 for the exposure of 15 s. The test tube containing 1 ml cell suspension and the plane transducer was placed in a water tank filled with degassed water during ultrasonic exposure. All experiments were randomly divided into four groups: 5-ALA-ultrasound treatment, ultrasound radiation alone and the controls including 5-ALA treatment alone and sham radiation. In the 5-ALA-ultrasound treatment the cells were pretreated by 5-ALA incubation before ultrasound radiation. The cells in ultrasound radiation alone were only radiated by ultrasound, but not incubated by 5-ALA. Those in 5-ALA treatment alone were only pretreated by 5-ALA incubation without ultrasound radiation and sham radiation without both ultrasound radiation and 5-ALA incubation. Other conditions of sham radiation were the same as the 5-ALA-ultrasound treatment, ultrasound radiation alone and 5-ALA treatment alone.

2.4. Cytotoxicity

The cytotoxicity of the K562 cells was assessed using the trypan blue (0.4%) exclusion test following the reports from Buldakov et al. [9] and Wang et al. [10]. The cells unstained by trypan blue were counted under light microscopy. The cell death rate was calculated using the following equation:

$$\text{cytotoxicity}\ (\%) = (\text{the unstained cell number of the control group} - \text{the unstained cell number of the treatment group})/\text{the unstained cell number of the control group} \times 100\%.$$

2.5. Mitochondrial membrane potential ($\Delta\Psi m$)

Mitochondrial membrane potential ($\Delta\Psi m$) were monitored using a Confocal Laser Scanning Microscopy (CLSM) with Rhodamine 123 staining. Briefly, the K562 cells were sensitized with 5-ALA (2 mM) for 4 h. The cells were then irradiated by ultrasound at the intensity of 0.46 W/cm^2 for the exposure of 15 s, and further incubated for 14 h. The Rhodamine 123 (dissolved in DMSO to produce a 1 mg/ml stock solution) (5 μg/ml) was added 30 min before cell harvesting. Washed cells were resuspended in PBS and observed using a CLSM (LSM 510, Zeiss, Esslingen, Germany) with the excitation setting at 488 nm, the fluorescence intensity of Rhodamine 123 were analyzed.

2.6. Mitochondrial morphological changes

Transmission electron microscopy (TEM) was used to observe mitochondrial morphological changes of the K562 cells 14 h after ultrasound exposure. The fixed cells were postfixed in 2% OsO_4, dehydrated in graded alcohol, and flat embedded in Epon 812 (Electron Microscopy Sciences, Fort Washington, PA). Ultrathin sections (100 nm) were prepared, stained with uranyl acetate and lead citrate, and examined under an electron microscopy (H-600; Hitche, Japan).

2.7. Statistical analysis

All values were the average of three experiments expressed as mean ± SD. Differences between data from two groups were assessed by t test and $P < 0.05$ was considered to be significant.

3. Results

3.1. Cytotoxicity of 5-ALA and ultrasound in the K562 cells

To assess the cytotoxicity of 5-ALA and ultrasound in chronic myelogenous leukemia cell line K562 cells, the treated cells were incubated for 24 h. The cells stained by trypan blue were counted. The cytotoxicity of the K562 cells in the presence of 2 mM 5-ALA after 15 s of exposure is shown in Fig. 1. The cell death of the K562 cells in sham radiation is 1.81 ± 0.13%. The cell death rate induced by ultrasound and 5-ALA treatment is 46.87 ± 4.09%, and 12.61 ± 2.63% with ultrasound radiation and no 5-ALA. The cell death rate induced by ultrasound and 5-ALA treatment was more significantly increased than that of ultrasound exposure alone ($P < 0.05$) and no effect on K562 cells following 2 mM 5-ALA treatment alone (1.27 ± 0.20%) ($P > 0.05$).

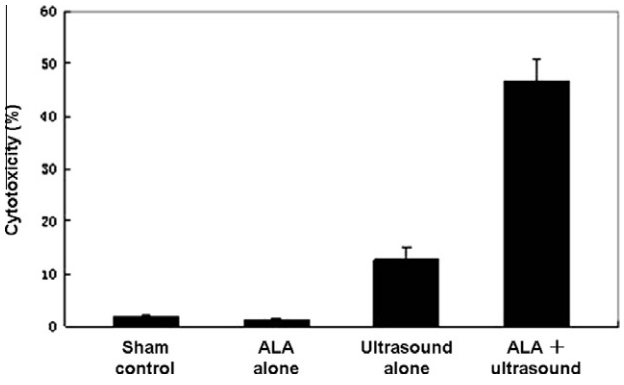

Fig. 1. The cytotoxicity of the K562 cells after different treatments. The controls include sham radiation and ALA alone. Sham control: the group with no treatment; ALA alone: the group with 2 mM 5-ALA alone; ultrasound alone: the group with ultrasound alone; ALA + ultrasound: ultrasound radiation plus 2 mM 5-ALA treatment.