Sonodynamic therapy with 5-aminolevulinic acid for angiosarcoma

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Introduction

Angiosarcoma is a rare malignant tumor of vascular origin that represents 2% of all soft-tissue sarcomas. This highly aggressive tumor spreads widely through the skin, recurs locally and metastasizes early. SDT with low-intensity ultrasound combined with a sonosensitizer may be a promising approach to cancer therapy. Sonosensitizers are known as 5-Aminolevulinic acid (5-ALA), bleomycin and photofrin, and others. The protocol of SDT has not been established. 5-ALA is an amino acid found in animals and plants. It is very important for energy generation in living bodies. 5-ALA has application for marketing approval of *in vivo* diagnostic agent for malignant glioma in Japan. 5-ALA is converted into protoporphyrin IX (PpIX) in mitochondria via biosynthesis pathway. PpIX is excreted from the mitochondria and becomes a sonosensitizer agent. Clinically this ultrasound device is used as a reduction of joint pain and muscle pain in the area of orthopedics. The hypothesized mechanisms of SDT are two pathways needing PpIX and not needing PpIX. First, the former is that ultrasound irradiation occurs cavitation bubble. Then, collapse of cavitation bubble produces the energy. The energy changes ground state of PpIX into excited state. When the excited state of PpIX returns to ground state, Reactive Oxygen Species (ROS) is produced. ROS production occurs apoptosis and necrosis. Next, the latter is that collapse of cavitation bubble produces ROS, mechanism stimulation and rise in temperature to cells. These reactions occur cell apoptosis and necrosis (Hiromoto T et al. Free Radical Biology and Medicine (2012)). We investigated the effect of *in vivo* SDT for angiosarcoma by low frequency ultrasound with 5-ALA.

Methods

To optimize concentration of 5-ALA:

Angiosarcoma cells (cell line: ISO-HAS-B) were cultured into a 24 well plate at a concentration of 10000 cells per well. After overnight incubation, 5-ALA was administered at each concentration (10mM, 7.5mM, 5mM, 2.5mM, 1mM) per well and incubated for 3 hours. Then all plates were washed three hours later and counted live cells using cell counting kit-8 and evaluated as cell viability 24 hours later.

To optimize power intensity and duty time of ultrasound device:

After overnight incubation, the cells were exposed to ultrasound. The ultrasound was used at wavelength of 1MHz, irradiation time of 2 minutes and power intensity of 3W/cm², 50% of duty time of maximum setting level of ultrasound. Duty time is time of applying irradiation time out of total irradiating time.

To show the anticancer efficacy for angiosarcoma by SDT:

We examined the efficacy of SDT by maximum setting level of ultrasound with a concentration of 2.5mM 5-ALA. Like previous experiment, angiosarcoma cell was incubated. After overnight incubation, the dishes were divided into following four groups. ① was no treatment group (ALA-, US-). ② was non-sonicated group (ALA+, US-). ③ was non-5-ALA group (ALA-, US+). ④ was the sonication + 5-ALA group (ALA+, US+). All plates were washed and then counted live cells with cell counting kit-8 24 hours later and observed in phase contrast image.

Caspase-3 staining for apoptosis:

The Angiosarcoma cells were incubated for 6 hours in a 24-well plate after the treatment of ultrasound and 5-ALA. Then, both the treated and control cells were stained with Caspase 3 staining.

Intracellular ROS measurement:

Intracellular ROS measurement was analyzed using an intracellular ROS assay with DCFH-DA (2,7-dichlorodihydrofluorescein diacetate) staining. DCFH-DA was added immediately after cells were exposed to ultrasound wave. Then, we detected fluorescence (480nm) immediately, 12 hours and 24 hours after sonication.

Figure 3. To show the anticancer efficacy for angiosarcoma by SDT:

The number of tumor cells was significantly decreased in SDT group compared with the other groups. pH adjusted. N=16 each. P < 0.05*.

Figure 4. Phase Contrast image:

The number of tumor cells was significantly decreased in SDT group.

Caspase / DAPI

Figure 5. Caspase-3 staining for apoptosis:

SDT group has more positive cells than other groups.

Results

Figure 1. To optimize concentration of 5-ALA:

The number of tumor cells was significantly decreased in over 5mM groups. This result shows that over 5mM concentration has cytotoxicity. From the above, 2.5mM was optimum concentration. pH adjusted. N=6 each. P < 0.05*.

Figure 2. To optimize power intensity and duty time of ultrasound device:

The number of cells was not significantly decreased in sonicated group compared with control group. We used the maximum setting level of ultrasound (power intensity of 3W/cm², 50% of duty time) because sonicated group has little cell damage to control group. pH adjusted. N=6 each.

Figure 6. Intracellular ROS measurement:

Compared with other group, ROS was increased in the SDT group. N=6 each. P < 0.05*.

Conclusions

SDT treatment has antitumor effect and induces reactive oxygen species. Then reactive oxygen species occurs apoptosis of angiosarcoma cells. We are going to examine the efficacy of SDT for *In Vivo* experiment.