Photodynamic therapy using 5-aminolevulinic acid in bone and soft tissue sarcoma

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INTRODUCTION: 5-Aminolevulinic acid (5-ALA) is a natural amino acid and a precursor of protoporphyrin IX (PpIX). PpIX functions as a photosensitizer (PS) and is accumulated higher in tumor cells because of the low activity of ferrochelatase, which is an enzyme that metabolizes PpIX to heme. PpIX is also excited by the exposure to light to exert an anti-tumor effect. Recently, the application of photodynamic therapy using 5-ALA was reported in patients with breast and brain cancer【1】. There are no reports about PDT using 5-ALA in bone and soft tissue sarcomas. We present an in vitro and vivo investigation of the outcome of PDT using 5-ALA on human osteosarcoma cell line (143B), mouse osteosarcoma cell line (LM8) and human fibrosarcoma cell line (HT1080). The aim of this study is to investigate the efficacy of PDT using 5-ALA on bone and soft tissue sarcomas, which may provide patient-specific prognosis for treatment outcomes【2】.

METHODS: The antitumor effect of PDT was investigated in vitro and in vivo using a human osteosarcoma cell line (143B), a mouse osteosarcoma cell line (LM8) and a human fibrosarcoma cell line (HT1080). Those cells were cultured in Minimum Essential Medium containing 10% fetal bovine serum in a humidified atmosphere and 5% CO_2 at 37°C. **In vitro study.** First, the cells were cultured (5×10^3 cells/well) in 96-well plates and after 24 hours, 0, 10, 100, 200 μg/ml of 5-ALA was administered and classified into the PDT (+) group and PDT (-) group. PDT group received the flash wave light (FWL, the light illumination frequency was 60 Hz and the pulse width was within 1ms. The energy was 15J and the illumination level was 1,000,000 lux.). The viability ratios of 143B, LM8 and HT1080 cells in each well were assessed by MTS assay at 0, 12, 24, and 48 hours. **In vivo study.** Tumor cells (2×10^6) were inoculated subcutaneously into the backs of BALBC mice (five-week-old males). After macroscopic tumor formation (5 mm in diameter), the following experiments were performed. The four groups were generated (n = 5) : The control group was non-treated, and the treatment groups were the illumination with FWL alone for 10 min, intraperitoneal administration of 5-ALA at 250 mg/kg alone and 5-ALA followed by illumination with FWL for 10 min (ALA-PDT) group. Body weight and tumor diameter were measured twice a week and the mice were killed on the 14th day after treatment, and the tumor tissue was resected for evaluation in all groups. All mice in every group survived to the study endpoint of 14 days. **Statistical analysis.** Significant differences between the two groups were compared using the Mann-Whitney U test, and more than three groups were compared using the Kruskal-Wallis chi-squared test. The statistical significance level was set at $p < 0.05$. Experiments were performed in accordance with the guidelines in the Declaration of Helsinki and the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing and Education.

RESULTS SECTION: In vitro, the 143B, LM8 viability of the 100 μg/ml and 200 μg/ml 5-ALA-PDT significantly inhibited cell proliferation at 24hours and 48hours, compared with the group of 0 and 10 μg/ml 5-ALA-PDT and PDT(-) groups. HT1080 viability of the 100 μg/ml and 200 μg/ml 5-ALA / 10 min FWL group significantly inhibited at 48hours, compared with the group of 0 and 10 μg/ml 5-ALA-PDT and PDT(-) groups. (Figure 1. a : 143B b : LM8 c : HT1080) In vivo, in all cell lines, a significant inhibitation in the tumor volume was observed in ALA-PDT group as compared to that in control, illumination with FWL alone and ALA alone groups. 1 (5-ALA- /L-), 2 (5-ALA-/10 min FWL) and 3 (250 mg/kg 5-ALA/L-) ($p < 0.05$). There were no significant differences among the three groups. (Figure 2. a : 143B b : LM8 c : HT1080)

DISCUSSION: In this study, we demonstrated the efficacy of PDT using 5-ALA treatment in osteosarcoma and fibrosarcoma cell lines. The 143B, LM8 and HT1080 viability of the 100 μg/ml and 200 μg/ml 5-ALA / 10 min FWL group was significantly lower than at 48hours after treatment. Several studies reported the efficacy of PDT using 5-ALA to breast cancer, skin cancer and so on. According to the findings of this study, PDT using 5-ALA may be a useful option for bone and soft tissue sarcoma.

SIGNIFICANCE/CLINICAL RELEVANCE: We show the efficacy of PDT using 5-ALA in bone and soft tissue sarcoma in vitro and in vivo at the first time. Clinical applications should be expected in the field of sarcoma.

REFERENCES:【1】G. Guney Eskiler, et al, *Photodiagnosis and Photodynamic Therapy* 31 (2020) 101854.【2】Yu, Wei, et al. *Oncotarget* 8.24 (2017): 39833.

Figure 1

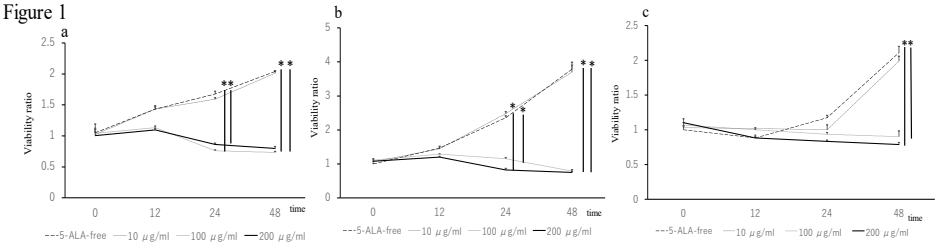

Figure 2