Photodynamic therapy (PDT) is a novel approach to cancer treatment that applies light and molecular oxygen in combination with a photosensitizing agent to selectively damage tumor tissue. This present study evaluated the effectiveness of 5-aminolevulinic acid (ALA) mediated PDT for inducing in vitro death in MG-63 osteosarcoma cells. To determine the accumulation of the photosensitizer (protoporphyrin IX, PpIX) in response to increasing ALA concentrations in the osteosarcoma cells, cells were treated with varying concentrations of ALA (0, 0.1, 0.25, 0.5, 1, 2, 5, 10 mM) for 4 and 24 h and then exposed to different light dosages (0, 0.5, 1.5, 10 J/cm²) using an LED array (λ= 636 nm). Cell viability was assessed using an MTT assay. It was found that the highest PpIX accumulation occurred in MG-63 cells treated with 0.5 mM and 1 mM ALA for 24 h. Consistent with these results, the greatest cell death was seen in cells treated with 0.5 mM and 1 mM ALA—there were more PpIX within those cells to generate a greater number of reactive oxygen species (ROS), the toxic element in the PDT mechanism. Overall, these findings suggest that ALA-mediated PDT significantly induces MG-63 cell death, supporting its potential use as a minimally invasive, alternative treatment of human osteosarcoma.

Background

Osteosarcoma is a metastatic primary malignancy of bone arising from the mesenchyme. It has few known genetic or environmental influences. It occurs most frequently around the knee between the ages of 10 and 20 and over 50. Current treatment involves chemotherapy and surgical removal, but 30–40% of the patients respond poorly (1). With the majority of patients being young, and with the amount that respond poorly to conventional treatment, there is room for the introduction of new techniques in the treatment. One such potentially new technique is photodynamic therapy.

Photodynamic therapy (PDT) is a treatment for cancer which utilizes a photosensitizer and a specific wavelength of light to produce reactive oxygen species (ROS) resulting in cell death and death (2). 5-aminolevulinic acid (ALA) is a produg favored for its lack of side effects (4). When treated with ALA, neoplastic cells accumulate the photosensitizer protoporphyrin IX (PpIX) in the mitochondria. PpIX is an intermediate in heme production in the cells, and when exposed to red light (635 nm), it results in ROS production (3). Single oxygen is believed to be the main cause of cell death, but due to its short life span within the cell (10-40 ms), it only damages the structures nearest to it, the mitochondria (2). Therefore, we hypothesize that ALA-mediated photodynamic therapy will induce cell death via mitochondrial mechanisms.

Results and Conclusion

PDT appears to be effective in killing MG-63 osteosarcoma cells. While PDT results were inconsistent with only 4 hours of ALA incubation; following 24 hour of ALA incubation significant cell death was observed. Cell death increased with increasing light exposure up to 10 J/cm², the maximum tested. The same trend does not appear to hold true for ALA concentrations. Cell death peaked when cells were treated with 0.5 mM ALA and were exposed to 10 J/cm² of light resulting in approximately 70% cell death. ALA concentrations higher than 0.5 mM produced less death, and this result is consistent with PpIX accumulation data. Cells accumulated the most PpIX when treated with 0.5 mM or 1 mM ALA for both the 4 and 24 hr incubation periods and PpIX accumulation decreased with ALA concentrations higher than 1 mM (figure 3). Less PpIX present in the cell would result in less ROS production and less cell damage and death, as observed in the PDT data. The decreased accumulation of PpIX may be due to cytotoxic effects of ALA. If ALA itself is killing cells then they would not be expected to accumulate PpIX. While our own cytotoxicity assays were inconclusive (data not shown), other studies have shown ALA to be cytotoxic at concentrations above 3 mM (5). The cytotoxicity of ALA may explain the results of PDT at the 5 and 10 mM ALA concentrations. At 5 and 10 mM ALA concentrations, cell death remained high relative to the decreased amount of PpIX accumulation, and this may be due to the combined effect of PDT and cytotoxicity. Therefore, the maximum effective dose of ALA for inducing cell death via PDT is in the range of 0.5 to 1 mM for at least 24 hours.

References