ACRIDINE ORANGE INCREASES RADIATION SENSITIVITY OF HUMAN CHONDROSARCOMA CELL LINE

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Introduction: Chondrosarcomas are malignant tumors of cartilaginous tissue and present a clinical challenge due to their resistance to chemotherapy and radiation. The most common treatment is surgical ablation, which does not always show favorable outcome. In one report, the 5-year survival rate for chondrosarcoma patients was 7% (1). Resistance to chemotherapy is believed to be due to the presence of multidrug resistance; however the reason for resistance to radiation is unclear. Acridine orange (AO) has been used to sensitize mouse osteosarcoma cells to low dose radiation. It is suggested that AO excitation by radiation causes release of singlet oxygen, which is toxic to living cells (2). The aim of the present study is to determine if addition of AO can similarly sensitize chondrosarcoma cells to low-dose radiation.

Methods: AO Exposure and Irradiation. Cells from an already established chondrosarcoma cell line (CS) were used for this experiment. The cells were cultured as a monolayer in growth medium as previously described (3). CS cells were cultured as monolayers in thirteen 100 mm tissue culture dishes at density of 250,000 cells/plate. Following a 15 minute exposure to AO at concentration of 0.5 µg/ml (AO-radiation group), the cells were irradiated with 0, 1, 2, 3 and 5 Gy of γ-rays using a 10,000 Ci cesium source. Controls were set up with cells treated with only radiation (1, 2, 3 and 5 Gy), cells treated with only AO (0.1, 0.25 and 0.5 µg/ml) and cells treated with neither radiation nor AO.

Colony Formation Assay. Immediately following the treatments, the cells were harvested using trypsin/EDTA. The isolated cells were seeded in 60 mm tissue culture dishes at a density of 1000 cells/dish. These cells were cultured for ten days in growth medium and kept in the darkness. The numbers of colonies containing greater than 50 cells were recorded after fixing with buffered formalin and staining with a mixture of Azure II and Methylene Blue dyes. Surviving fraction was calculated as previously described (3). CS cells were cultured as monolayers in growth medium established chondrosarcoma cell line (CS) were used for this experiment.

Essential Results: After exposure to various conditions, cell viability was investigated by colony formation assay. Results of colony formation assay were used to establish survival curve for the CS cells as shown in Figure 1. This figure indicates that addition of AO sensitizes the CS cells to the radiation in a dose dependent manner. It is also observed that AO at doses of 0.1, 0.25 and 0.5 µg/ml without the radiation cause minor reduction of the survival fraction of CS cells.

Discussion: Photodynamic therapy (PDT) involves use of a localized photosensitizer that is excited by light to cause damage and subsequent cell death. PDT is an encouraging new technique used in treatment of bladder, esophageal and lung cancers as well as in nonmalignant diseases. AO is a cell-permeable, fluorescent dye that interacts with DNA and RNA of living cells by intercalation or electrostatic attraction. Long exposures to AO (about 24 hours) cause mitotic arrest in normal chondrocytes in cell culture (4). AO is used in histochemistry for diagnosis, classification, and prognosis of neoplasms and has been experimentally used in PDT of superficial tumors such as bladder, gastric and epithelial cancers because of its nature as a photosensitizing agent. Recent results published have demonstrated that AO can be excited by low-dose X-ray as well as visible light, making it feasible for use in deep structures of the body (5).

Results of our in vitro study demonstrate that a treatment regimen of AO and low-dose radiation was effective in eliminating or stopping division of the CS cells. In cells cultures treated with AO and 3 or 5 Gy, there were many single cells and small colonies. This is an indication that the cells did not necessarily die as a result of the treatment but were incapable of cell division. In future studies, the combined AO and radiation therapy can be used to treat an animal model of chondrosarcoma to show that it is effective both in vitro and in vivo. There is also potential to use various other photosensitizers for treatment of chondrosarcoma and overcome the radiation resistance characteristic of these tumors.

Figure 1: Survival fraction of CS cells treated with AO alone (0, 0.1, 0.25 and 0.5 µg/ml), radiation alone (0, 1, 2, 3 and 5 Gy) and combination of AO and radiation as measured by colony formation assay.

References