IN VIVO EFFECT OF ACRIDINE ORANGE ENHANCED BY MINIMAL DOSE OF RADIATION ON MOUSE OSTEOSARCOMA MODEL

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INTRODUCTION: Although it is well-known that acridine orange has a strong photodynamic inactivation effect in vitro and in vivo on various tumor cells, light beam for excitation does not reach to deep sites. In order to improve this problem, we developed a combination therapy of acridine orange (AO) and low dose radiation and indicated that this therapy had a strong cytocidal effect in vitro on mouse osteosarcoma cells. In this study, we undertook to clarify the in vivo effect of this combined therapy on mouse osteosarcoma model.

MATERIALS AND METHODS: Mouse osteosarcoma cells obtained from radiation-induced MOS cell line was used in the study. The deeply freezed MOS cells were cultured in the medium (DMEM) containing 10% FBS in 5% CO2 atmosphere at 37°C. The isolated 5 x 10^6 cells by tripsinization were subcutaneously inoculated in the right lower limb of mice. When the tumor grew up to 8 mm at maximum diameter, 10µg/ml of AO was intraperitoneally injected in each mouse. X-ray irradiation to these mice (n= 12) was performed by the linear accelerator (Lineac: Mitsubishi Elec. Co.). Applied dose of X-ray was 5 Gy, which was found to be most effective dose by in vitro study. Conditions in the irradiation were at 100 cm of distance from focus to specimen, 1.40 Gy/min of irradiation ratio, 24.3°C of temperature and 1021.8hp of atmospheric pressure. Tumor volume (maximum diameter x minimum diameter^2 / 2) was measured with a vernier micrometer at every 3 days after radiation. In control study groups, mice having tumor were treated without AO and radiation (n=15), with AO alone (n=13), and with radiation alone (n=12).

RESULTS: All of the control groups showed a constant increase of tumor volume up to 30 days. There was statistically (student T test) no significant difference of tumor growth rate among these control groups. However, in the treated group by combination therapy of AO and radiation, the tumor growth rate was significantly inhibited, compared to that of the control groups (p<0.001) (Fig.1). The tumors treated with radiation or AO alone histologically showed active cell growth with osteoid formation (Fig.2), whereas tumors treated with AO and radiation demonstrated massive tumor necrosis, despite a few cells were still alive (Fig.3). However, the treated tumors showed regrowth after more than 30 days.

DISCUSSION: Results of the study revealed that combination therapy of AO and low dose radiation is effective to inhibit in vivo tumor growth of mouse osteosarcoma. Therefore, it is suggested that AO may be a novel radiosensitizer on osteosarcomas. However, it is difficult to obtain total cell death by single course of the therapy. If we can apply this technique to human osteosarcomas, unresectable tumors localized in pelvic bone or spine, or multiple pulmonary metastatic tumors may be good indication, although we have to clarify the optimum conditions of frequency of the therapy, doses of radiation and AO.

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