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Radiosensitizing Effects of Adriamycin on Hypoxic Cells

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Recent advances in clinical radiobiology have revealed that hypoxic cells in malignant tissues are highly radioresistant to X or Gamma rays. Many chemical agents have been introduced to reduce this radioresistance of the hypoxic cells. Among these drugs, Adriamycin which was recently developed as an anti-cancer agent was reported to have a radiosensitizing effect in vivo. In this paper the toxicity of Adriamycin on hypoxic cells and the possibility of the drug as a radiosensitizer in vitro are discussed. A concentration of 0.1 μg/ml of Adriamycin showed a slight toxic effect and a marked radiosensitizing effect on experimentally produced hypoxic HeLa S-3 cells.

(Key Words: Radiation, Sensitizer, Hypoxic cells)

INTRODUCTION

In radiotherapy for malignant tumors, the existence of a few percent of hypoxic cells in tumor tissues has recently come into question (19). It has been found that hypoxic cells are twice as radioresistant as aerobic cells to X or Gamma rays which are now widely used clinically (7, 8). Therefore, the question is how these hypoxic cells are affected by radiation. To solve this problem physically, high LET radiations such as neutrons, pi mesons and heavy charged particles with a low oxygen enhancement ratio (OER) have been applied in radiotherapy (3, 4, 10). In addition, a hypoxic cell radiosensitizer has been investigated to solve this problem chemically (1, 16). The concept for this sensitizer is to let hypoxic cells have the same radiosensitivity as that of aerobic cells by taking up chemical compounds. Several reports on hypoxic sensitizers have been published, and clinical trials have been carried out (5, 9). Among the anti-tumor agents, there are some drugs such as Actinomycin D and Bleomycin which show radiosensitizing effects (14, 21, 22, 11, 17). They have been applied clinically, although neither of them are sensitizers for hypoxic cells. This report describes the sensitizing effects of Adriamycin on in vitro hypoxic cells.

MATERIALS AND METHODS

Cell culture

HeLa S-3 cells (courtesy of Dr. Inui; Section of Cell Biology and Cyto-

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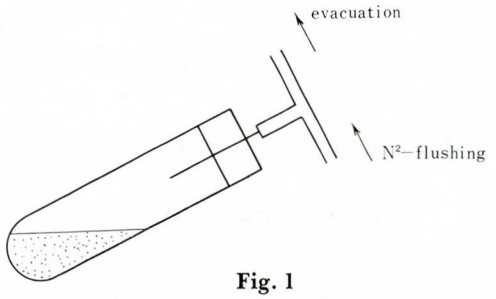

Fig. 1

Table 1.

Times for hypoxygenation (min.)	Procedure
5.0	evacuation *
10.0	1st N_2-flushing **
12.5	evacuation
15.0	2nd N_2-flushing
17.5	evacuation
20.0	3rd N_2-flushing
22.5	evacuation
25.0	4th N_2-flushing

* Using a laboratory rotary vacuum pump.
** A nitrogen gas mixture containing 5% CO_2 was used. The flow rate was 1.5 L/min.

genesis, Bilogical Research Center, J.T.S.) were used in this study. The cells were grown attached to the surface of plastic tissue culture flasks (Nunk) in Eagle's minimum essential medium (Nissui) supplemented with 10% new born calf serum (Hokuiken). They were incubated at 37°C in an atomosphere of 95% air and 5% carbon dioxide at 100% humidity. The cells were subcultured every three to four days. A solution of 0.1% trypsin (Difco) containing 0.02% EDTA was used to detach them from the plastic surface for subculturing.

Chemical

Adriamycin produced by Farmitalia S.P.A. (Montedison Group) was dissolved in sterile saline and diluted to an appropriate concentration in complete growth medium immediately prior to the experiments.

Hypoxygenation

Cell suspensions for experimental use were prepared from log phase monolayer cultures; 1 ml of cell suspension ($2.5 - 5 \times 10^4$ cells/ml) was incubated for 12 hours in Falcon plastic tubes. Hypoxygenation was carried out according to the procedure shown in Fig. 1 and Table 1.

Irradiation

Irradiation was performed with Gamma rays using a cobalt 60 teletherapy unit (Theratron 780) or with 10 MV X rays using a linear accelerator (Varian). Cell suspensions in Falcon tubes were placed in a Mix Dp Phantom, and varying doses of X or Gamma rays were applied. The absorbed dose was calculated by an ionizing chamber (AE—130LS Applied Science Co., Ltd., Japan) and TLD dose meter (Harshow Co., Ltd., USA).

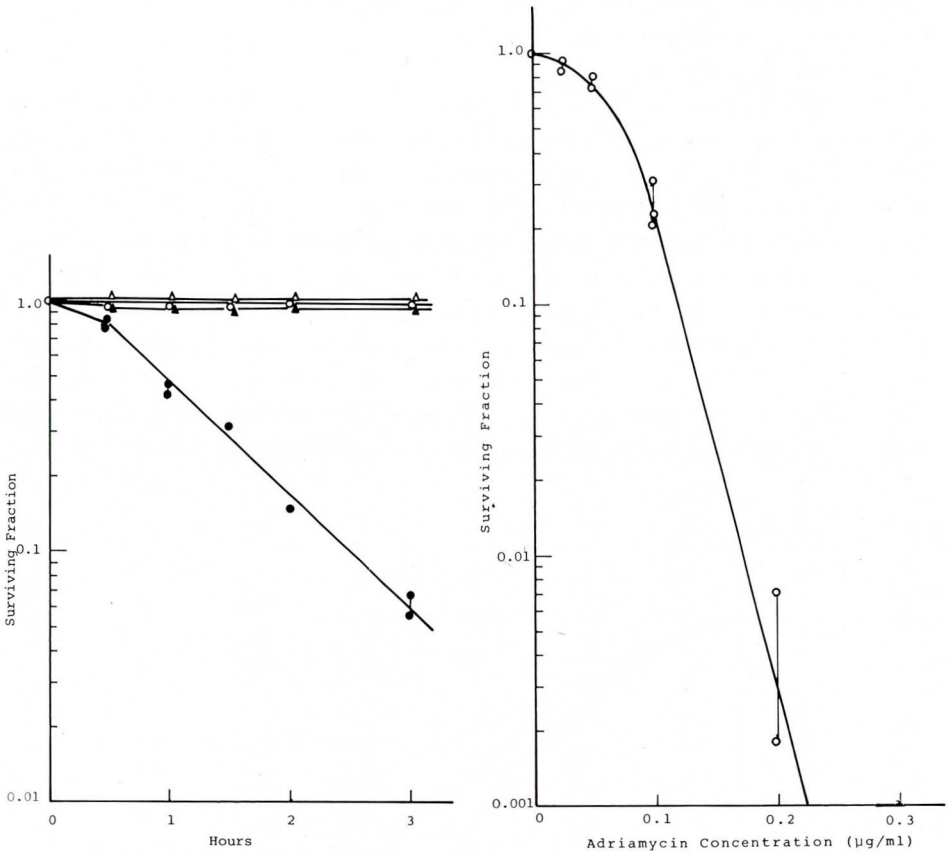

Fig. 2 Survival of growing HeLa S-3 cells exposed to various concentrations of Adriamycin as a function of exposure time. The drug was added to culture media 20 hrs after plating. Survival of the cells was determined by the colony formation method.
△—△ 0.001μg/ml ○—○ 0.005μg/ml
▲—▲0.01 μg/ml ●—● 0.1 μg/ml

Fig. 3 Survival of HeLa S-3 cells incubated with various concentrations of Adriamycin for one hour. Exposure conditions were the same as in Fig. 2.

Cell survival

Cell survival was determined by the colony formation method. Cell suspensions in plastic petri dishes (Nunc) 60 mm in diameter were incubated at 37°C for 12 to 14 days. Colonies formed were fixed with methanol and stained with Giemsa (Merck). Plating efficiency (PE) of the control was 75 to 80%.

RESULTS

Toxicity of Adriamycin

Toxicity of Adriamycin on the cells was determined as shown in Fig. 2. In the case of treatment for three hours, it was found that there was no toxicity at concentrations of 0.001 μg/ml to 0.01 μg/ml. The survival decreased to 0.06 with 0.1 μg/ml for three hours of exposure. In incubation with Adriamycin of increasing concentrations for one hour, it was found

that the survival decreased to 0.25 with 0.1 μg/ml and to less than 0.01 with 0.2 μg/ml as shown in Fig. 3.

Radiosensitizing effects

Survival curves of HeLa S-3 cells were obtained as a function of radiation dose for both aerobic and hypoxic cells. It was found that D_0 was 209 rads (n = 2.5) for aerobic cells and 578 rads (n = 1.1) for hypoxic cells. Survival rates were investigated after Adriamycin was added to the culture at a concentration of 0.1 μg/ml. It was suggested from the previous experiments that the concentration of 0.1 μg/ml has little influence on the survival rate after about one hour of exposure. The results are shown in Fig. 4. There was little difference in survival rates between aerobic cells with and without Adriamycin, but for hypoxic cells there was a big difference. The value of D_0 decreased to 278 (n = 2.3), and it was found that Adriamycin showed radiosensitizing effects on the hypoxic cells.

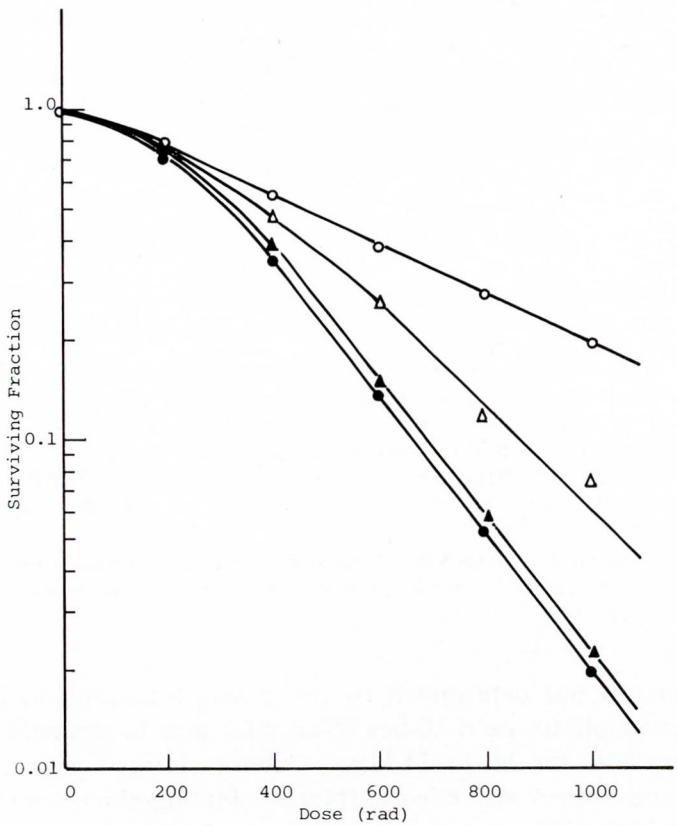

Fig. 4 Cell survial as a function of radiation dose for HeLa S-3 cells exposed to 0.1 μg/ml of Adriamycin for one hour during irradiation.
○—○ Hypoxic △—△ Hypoxic in 0.1 μg/ml Adriamycin
▲—▲ Aerobic ●—● Aerobic in 0.1 μg/ml Adriamycin

DISCUSSION

Adriamycin (14-hydroxydaunomycin) was first discovered in culture media of Streptomyces peuceticus var. caesius in 1967 and was found to

have anti-tumor activity due to the inhibition of DNA synthesis (2). It has been widely used clinically, especially for the treatment of malignant lymphomas, bronchogenic carcinomas and gastrointestinal tract malignancies. To determine the radiosensitizing activity of Adriamycin on hypoxic cells, it is necessary to establish experimental hypoxic conditions. Two methods have been adopted in treating hypoxic cells, i.e. spheroids (V-79 cells) formed artificially, and oxgen content decreased by gas exchange (6,18,12,13). The latter method was investigated in the present study. Hypoxic conditions were achieved by repeated evacuation and flushing with N_2 gas containing 5% CO_2. The dose modifying factor (DMF) (D_0aerated/D_0hypoxic) of HeLa S-3 cells was 0.36. When the radiosensitizing effect of Adriamycin was examined, no radiosensitizing effect was observed at the level of 0.1 μg/ml under aerobic conditions, but it was observed under hypoxic conditions. The DMF with Adriamycin was 0.75. Action mechanisms of Adriamycin as a radiosensitizer might be 1) metabolic action leading to reoxygenation of hypoxic populations or 2) inhibition of DNA repair of radiation induced strand breaks. In the former case, in vitro experiments are not suitable for testing the radiosensitizing activity of the drug and in the latter case, aerobic cells would also be sensitized by Adriamycin. However in this experiment, it was found that aerobic cells are hardly sensitized. Therefore, there are many interesting points, such as whether or not DNA repair of radiation induced strand breaks is inhibited in hypoxic cells and whether or not inhibition of DNA repair just after radiation treatment influences the final colony formation ability? Adriamycin was used at a concentration of 0.1 μg/ml, and the DMF was 0.75 in this experiment, but if a less toxic concentration is used, how will the sensitizing effect change? How is the DMF changed by neutrons which are considered to impair both hypoxic and aerobic cells without distinction? These points will be investigated further.

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