Oral

EFFECT OF A PORPHYRIN COMPOUND ON CULTURED CELLS UNDER X-RAY EXPOSURE

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Introduction

Among multifunctional cyclic tetrapyrrole family, porphyrin compounds serve as critical elements in oxygen transport and photosynthesis, which are indispensable to support vital activities. They have been used in many industrial applications such as functional materials, catalysts, nano-sized materials, as well as in medical applications, with a growing expectation to extend their envelope. To date, a hypothesis has been proposed that x-ray radiation activates porphyrin derivatives, resulting in a yield of red radio luminescence[1]. On the other hand, oncotropic hematoporphyrin derivatives have been used in photodynamic therapy of cancer treatment, in which they work as a photosensitizer on red laser irradiation. To investigate the possibility as a sensitizer also in x-ray radio therapy. we studied the effect of oncotropic porphyrin compound on cultured cells excited by x-ray irradiation.

Materials and Methods

ROS Generation Test

A series of x-ray exposure tests were conducted to measure the amount of ROS generation from a porphyrin compound. Concentrations of Protoporphyrin IX ($C_{34}H_{34}N_4O_4$ MW 562.66: PpIX) in a 200 μl phosphate-buffered saline (PBS) were varied at 0.3, 1.0, 3.0, 10.0 and 30.0 ug/ml in wells of 96 multi-well plates. To measure the ROS generation 50 μM dihydroethidium (Invitrogen) was added in each well [2]. Prior to x-ray exposure test, absorbed dose rate for water was measured by a free air ionization chamber with a 3 cc volume (RAMTEC 1500-DC300, Toyomedic. Ltd.) under the above operating condition as a function of distance from the focal point. Exposure period was controlled for 1.0, 2.0, 3.0, 5.0 and 10.0 min so that the integrated absorbed dose for water at the well surface became 1.0, 2.0, 3.0, 5.0 and 10.0 Gy.

Fig. 1 shows the relative values of the fluorescence intensity for each material against PBS alone as control. Effects of radioactivation of water and surrounding light were cancelled by subtracting the fluorescence intensity in control wells. ROS generation was particularly noted at high concentration of 30 ug/ml for PpIX.

CFU- Assay

A normal mammalian cell tends to form a colony of several tens of cell aggregation when cultured in appropriate conditions. This colony-forming unit (CFU) has been used to evaluate the cell proliferation function as a result of radiation damage. Following the ROS generation test, in vitro studies were carried out to estimate the effect of generated ROS on the viability of HeLa cells (obtained from RIKEN Cell Bank).

Fig. 2 shows the ratio of colonies of cells irradiated at different doses of x-ray to those of control cells incubated without treatment of PpIX. It has been reported that the percent survival of irradiated cells is linearly correlated to the absorbed dose in semi-log plot [3]. Our result of irradiated cell viability without PpIX agrees with this tendency. For all x-ray doses, the colonies of the cells treated with PpIX were less than those without PpIX, suggesting that the colony-forming ability is impaired further by PpIX in addition to radiation damage.

Results

Based on the experimental results, we found that PpIX generates ROS upon x-ray irradiation, and enhances cell damage by ionizing radiation. Oncotropic PpIX is uptaken into cytosol of

cells in culture and tends to localize near a nucleous(Fig.3). A possible mechanism of enhanced cell damage is through a synergistic effect of direct DNA strand break and cytosolic damage caused by PpIX excited by x-ray radiation, suggesting that PpIX may serve as a promising radiosensitizer. We expect that PpIX and its derivatives can become more effective by enhanced oncotropic property and increased excitation by x-ray radiation.

References:

[1] Bistolfi F., Panminerva Med, 42(1) (2000), 69-75.
[2] Zuo L, et al., Am J Physiol Cell Physiol., 279 (2000), C1058–66.
[3] Elkind MM, Sutton H., Radiat Res., 13(4) (1966), 556–93.

Figures:

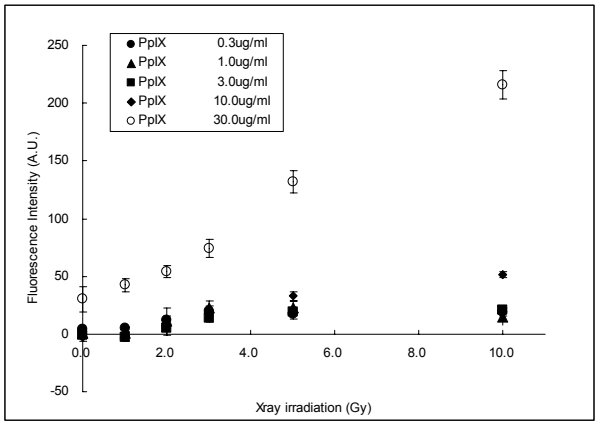

Fig.1 Fluorescence intensity of ethidium generated by PpIX at different x-ray doses. The solution was excited at 465 nm, and its fluorescence intensity at 585 nm was measured by a fluorescence microplate reader (infinite M200, TECAN).

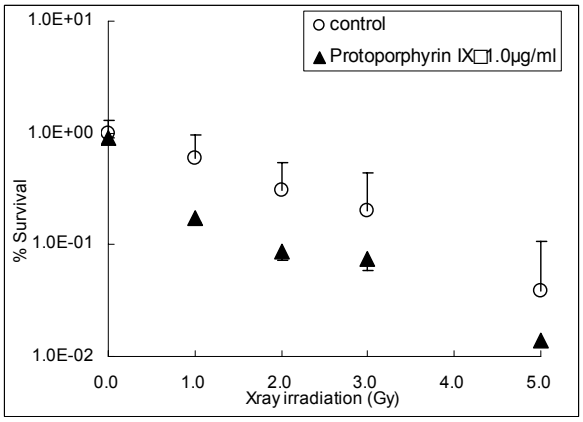

Fig. 2 Surviving fraction of HeLa cells loaded with PpIX (1.0ug/ml) under different doses of x-ray. The ratio was taken against the number of control cells incubated without treatment of PpIX. Colony formation was measured 9 days after the x-ray irradiation.

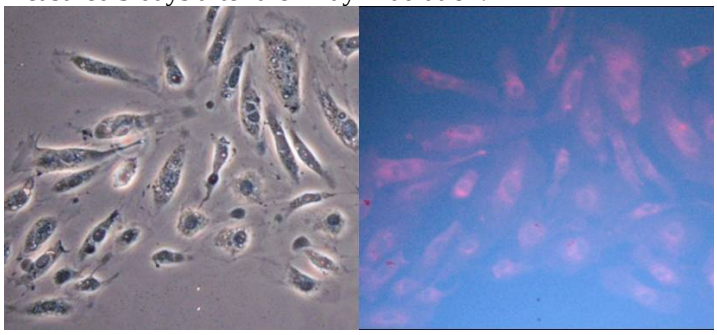

Fig. 3 Uptake and distribution of PpIX in cytosol and around a nucleus ~~of HeLa cells~~ were observed by a fluorescence microscope after one hour incubation in culture medium mixed with PpIX, which has a fluorescence peak at 630nm.