RADIOSENSITIZING EFFECT OF PROTOPORPHYRIN IX WITH CARBON ION BEAM AGAINST MOUSE MAMMARY BREAST TUMOR CELL

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ABSTRACT: 5-Aminolevulinic acid (ALA), a precursor of protoporphyrin IX (PpIX), has been utilized as a potent sensitizer for photodynamic therapy (PDT) of cancer through production of reactive oxygen species (ROS). ALA also has been proposed as a possible radiosensitizer with low linear energy transfer (LET) radiation because antitumor activity of low-LET radiation depends on ROS mainly. In this paper, we investigated the radiosensitizing activity of PpIX in carbon ion irradiation as a high LET radiation. PpIX was detected in an EMT6 mouse mammary breast tumor cells even at 24 h post treatment. The cytotoxicity of PpIX against EMT6 cells was IC50 = 48.0 μm. The combined treatment of 1.0 μm of PpIX with 2 Gy of carbon ion irradiation (290 MeV/nucleon, 85.1 keV/μm) showed a more potent antitumor activity than carbon ion irradiation alone. We demonstrated that PpIX showed a radiosensitizing effect with carbon ion beam against mouse mammary breast tumor cells.

KEYWORDS: *Carbon Ion Beam, Protoporphyrin IX, Aminolevulinic Acid, Radiosensitization*

1.0 INTRODUCTION

5-Aminolevulinic acid (ALA), a precursor of protoporphyrin IX (PpIX), has been utilized as a potent sensitizer for photodynamic therapy (PDT) for cancer through production of reactive oxygen species (ROS) by photoexcitation of PpIX [1-3]. ALA and PpIX also have been proposed as a possible radiosensitizer with low linear energy transfer (LET) radiation [4, 5], because antitumor activity of low-LET radiation depends on ROS mainly. On the other hand, the antitumor effect of high LET radiation, such as heavy particle beam and neutron beam, does not depend on ROS. However, we speculated that PpIX has possibility to generate ROS in response to heavy particle beam because heavy particle beam also includes a little of low LET radiation. Therefore, we investigated the radiosensitizing activity of ALA in carbon ion irradiation.

2.0 METHODOLOGY

Intracellular concentration of PpIX was measured as follows; EMT6 cells (1 × 105 cells/mL) were treated in 10 μM of PpIX and collected cells in each time and detected the fluorescence of PpIX (emission: 410 nm, excitation: 630 nm).

Antitumor activity of PpIX was measured as follows; EMT6 cells (1 × 103 cells/mL) were treated in each concentration of PpIX at 24 h and cell viability was measured by WST-1 assay.

In vitro radiosensitization was measured in EMT6 single cells. PpIX was added to an EMT6 cell (5 × 105 cells/mL) suspension at a dose of 1 μM in cell culture flasks. The cells were irradiated with a 290 MeV/nucleon (85.1 keV/ μM) carbon beam in HIMAC at the National Institute of Radiological Sciences (NIRS, Chiba) by 1-2 Gy at 24 h post treatment of PpIX. After irradiation, colony formation assays were performed. Surviving fraction (SF) was determined from the ratio of the number of colony of control.

3.0 RESULTS

3.1 Intracellular Uptake of PpIX

We evaluated the intracellular uptake of PpIX using EMT6 cells. PpIX was detected in an EMT6 mouse mammary breast tumor cells at 1 h post treatment and showed a maximum concentration at 24 h post

treatment (Figure 1). Therefore, we decided carbon ion irradiation time at 24 h post treatment.

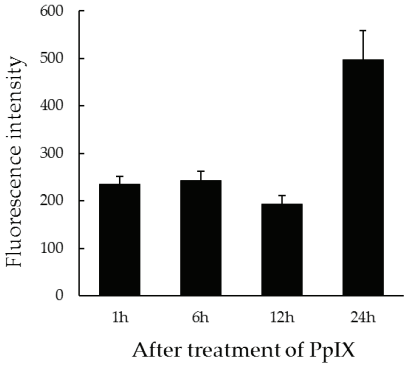

Figure 1: Intracellular concentration of PpIX

3.2 Cytotoxicity of PpIX

Next, we evaluated the cytotoxicity of PpIX against EMT6 cells. As shown in Figure 2, the cell viability rate of 1 μM of PpIX group at 24 h post treatment was 99.7 %, indicating that 1 μM of PpIX showed no cytotoxic effect on EMT6 cells under our experimental conditions. Obviously, cell viability was concentration-dependently inhibited by equal more than 3 μM of PpIX. The IC50 value of PpIX was 48.0 μM.

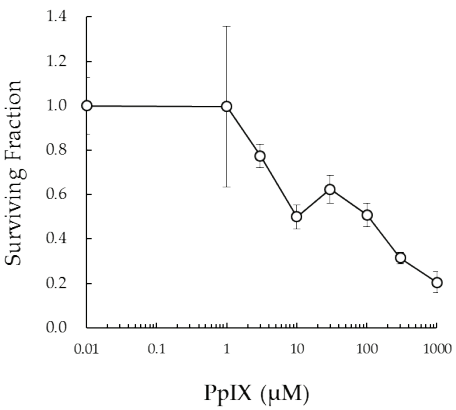

Figure 2: Cytotoxicity of PpIX against EMT6 cells

3.3 Radiosensitizing Activity of PpIX

Figure 3 shows the radiosensitizing effect of PpIX against EMT6 cells to single-dose carbon ion irradiation. EMT6 cells treated with 1.0 ◎M of PpIX 24 hours before 2 Gy of carbon ion irradiation showed higher radiosensitivity (SF = 0.071) than control cells (SF = 0.196). However,

there were no differences in the 1 Gy of single-dose carbon ion irradiation between the PpIX treated group and the control (SF = 0.421 and SF = 0.479, respectively).

K. Matsumoto et al., reported that hydrogen peroxide generation was occurred by relatively high-dose of carbon ion irradiation [6]. Additionally, J. Zeng et al., reported that PpIX can catalyze conversion of hydrogen peroxide into singlet oxygen which having a potent antitumor activity [7]. Therefore, we speculated that PpIX generated singlet oxygen via carbon ion beam-induced hydrogen peroxide.

On the other hand, C. Sun et al., reported that carbon ion beams induce hepatoma cell death by NADPH oxidase-mediated mitochondrial damage [8]. We suggested that PpIX enhance the mitochondrial damage by carbon ion irradiation because PpIX accumulate mitochondria [9].

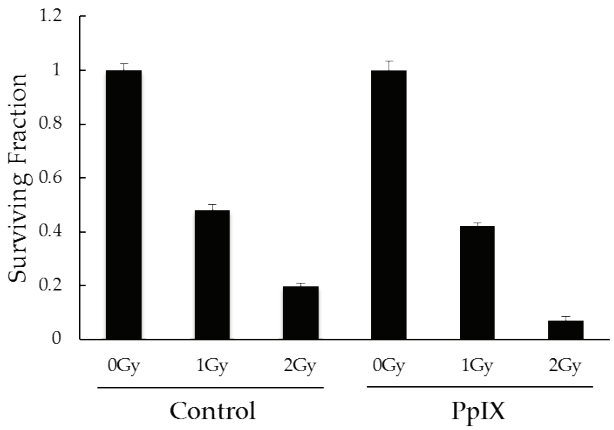

Figure 3: Radiosensitizing activity of PpIX with carbon ion beam

4.0 CONCLUSIONS

We demonstrated that PpIX showed a radiosensitizing effect with carbon ion irradiation against mouse mammary breast tumor cells.

ACKNOWLEDGMENTS

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REFERENCES

[1] X. Yang, P. Palasuberniam, D. Kraus D and B. Chen, "Aminolevulinic Acid-Based Tumor Detection and Therapy: Molecular Mechanisms and Strategies for Enhancement." *Int. J Mol. Sci., Vol. 16*, No. 10, pp. 25865-80, Oct. 2015.

[2] S. L. Marcus, R. S. Sobel, A. L. Golub, R. L. Carroll, S. Lundahl and D. G. Shulman, "Photodynamic therapy (PDT) and photodiagnosis (PD) using endogenous photosensitization induced by 5-aminolevulinic acid (ALA): current clinical and development status." *J. Clin. Laser Med. Surg., Vol. 14*, No. 2, pp. 59-66, Apr. 1996.

[3] P. Cappugi, P. Campolmi, L. Mavilia, F. Prignano and R. Rossi, "Topical 5-aminolevulinic acid and photodynamic therapy in dermatology: a minireview." *J. Chemother., Vol. 13*, No. 5, pp. 494-502, Oct. 2001.

[4] T. Kitagawa, J. Yamamoto, T. Tanaka, Y. Nakano, D. Akiba, K. Ueta and S. Nishizawa, "5-Aminolevulinic acid strongly enhances delayed intracellular production of reactive oxygen species (ROS) generated by ionizing irradiation: quantitative analyses and visualization of intracellular ROS production in glioma cells in vitro." *Oncol. Rep., Vol. 33*, No. 2, pp. 583-90, Feb. 2015.

[5] Y. Kamada, Y. Murayama, K. Harada, M. Nishimura, Y. Kondo, H. Konishi, R. Morimura, S. Komatsu, A. Shiozaki, Y. Kuriu, H. Ikoma, M. Nakanishi, D. Ichikawa, H. Fujiwara, K. Okamoto, C. Sakakura and E. Otsuji, "Radiosensitizing effect of 5-aminolevulinic acid (5-ALA) in Colon cancer." *Gan To Kagaku Ryoho, Vol. 41*, No. 12, pp. 1608-10, Nov. 2014.

[6] K. Matsumoto, I. Aoki, I. Nakanishi, A. Matsumoto, M. Nyui, K. Endo and K. Anzai, "Distribution of hydrogen peroxide-dependent reaction in a gelatin sample irradiated by carbon ion beam." *Magn. Reson. Med. Sci., Vol. 9*, No. 3, pp. 131-40, Sep. 2010.

[7] J. Zeng, Q. Sun, J. Su, J. Han, Q. Zhang and Y. Jin, "Protoporphyrin IX catalyzed hydrogen peroxide to generate singlet oxygen." *Int. J. Clin. Exp. Med., Vol. 15*, No. 8, pp. 6829-34, May. 2015.

[8] C. Sun, Z. Wang, Y. Liu, Y. Liu, H. Li, C. Di, Z. Wu, L. Gan and H. Zhang, "Carbon ion beams induce hepatoma cell death by NADPH oxidase-mediated mitochondrial damage." *J. Cell Physiol., Vol. 229*, No. 1, pp. 100-7, Jan. 2014.

[9] M. E. Koller and I. Romslo, "Uptake of protoporphyrin IX by isolated rat liver mitochondria." *Biochem. J., Vol. 188*, No. 2, pp. 329-35, May. 1980.