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ARTICLE

Radiobiological characterization of neutron irradiation field of UTR-KINKI for the research utility toward boron neutron capture therapy: Cell killing effect and its enhancement by 4-borono-L-phenylalanine

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Purpose: Boron neutron capture therapy (BNCT) is shown to be effective on tumors that are refractory to conventional radiotherapies, such as photon and proton therapy. While the basic studies and clinical trials have been conducted using research reactors, the accelerator-based systems are being installed in the hospital. Here we evaluated the cell killing effects of neutron and its augmentation by 4-borono-L-phenylalanine (BPA) in the research reactor UTR-KINKI. Materials and methods: Three human cell lines, *i.e.*, submandibular gland HSG, melanoma A375 and squamous cell carcinoma SAS were used in this study. Cells were irradiated for 1 to 4 h at the center of UTR-KINKI, which was operated at 1 W output. The dose rates for neutron and γ-ray were reported to be 0.214 Gy h^{-1} and 0.196 Gy h^{-1}, respectively. The thermal neutron flux was 1.07×10^7 cm^{-2} s^{-1}. BPA was added to the culture media at the boron concentration of 12.5, 25 or 50 ppm. Cell survival was measured by colony formation assay. For the quantitative analysis, the cell survival curve was fitted to simple exponential function, *i.e.*, $\ln S.F. = -aT$, where S.F. and T are surviving fraction and irradiation time, respectively, and "*a*" is a constant. Sensitivity enhancement ratio (SER) was obtained as the ratio of "a" value with BPA to that without BPA. Results: Surviving fraction of HSG, A375 and SAS after irradiation for 3 h with 0.64 Gy of neutron and 0.59 Gy of γ-ray was 0.24, 0.21 and 0.40, respectively. BPA significantly decreased the survival of HSG, A375 and SAS cells in all the conditions examined. SER of 50 ppm boron concentration of BPA was 2.58, 2.13 and 3.71 for HSG, A375 and SAS cells, respectively.
Conclusion: The present study demonstrated the cell killing effects and its enhancement by neutron capture using BPA in UTR-KINKI of the output as small as 1 W. This reactor is shown potentially useful for the basic researches toward BNCT, such as the development of new boron-containing, neutron-capturing compounds and exploration for biomarkers.

Keywords: ***neutron; cell; boronophenylalanine (BPA); boron neutron capture therapy (BNCT); UTR-KINKI***

1. Introduction

Boron neutron capture therapy (BNCT) is shown to be effective on tumors that are refractory to conventional radiotherapies, such as photon and proton therapy [1]. The theoretical principle of BNCT was first proposed by Lockner in 1936. A stable boron isotope, ^{10}B, has an extraordinary reactivity with thermal neutron and produces alpha particle and ^{7}Li nucleus. Both of the alpha particle and ^{7}Li nucleus have a very short path, which is mostly equivalent to the diameter of a cell, and high linear energy transfer (LET), which exerts high biological effectiveness. Thus, BNCT enables effective killing of cancer cells with minimal effects on the surrounding normal cells, if ^{10}B is selectively incorporated to cancer cells. At present, two ^{10}B-containing compounds are used clinically: sodium borocaptate (BSH) and 4-borono-L-phenylalanine (BPA). The development of ^{10}B-containing compounds, which show more selective and efficient accumulation in cancer cells, are anticipated.

While basic studies and clinical trials have been conducted using research reactors, such as KURRI in

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Kyoto University, the accelerator-based systems are being installed in the hospital. Nonetheless, because of the stability and reproducibility of the neutron irradiation field, the research reactors would be useful for basic researches, such as the development of boron-containing, neutron-capturing compounds and exploration for biomarkers. UTR-KINKI in KINDAI University is a small-sized research reactor with a maximal output of 1 W [2] and has been used for education of nuclear reactor physics and biological researches, using fruit fly, soybean, mice and human tissue xenograft in mice [3-6]. Here we examined the effects of radiation of UTR-KINKI in terms with killing of cultured cells and its enhancement by BPA.

2. Materials and methods

2.1. Cell culture

Human salivary gland tumor HSG cell and human squamous cell carcinoma SAS cell were obtained from Japanese Collection of Research Bioresources Cell Bank (JCRB, Ibaraki, Osaka, Japan). Human melanoma A375 cell was obtained from American Type Culture Collection (Manasaas, VA, USA). Minimal essential medium (MEM), Dulbecco's modified Eagle medium (DMEM) and DMEM/Ham's F12 medium were used for HSG, A375 and SAS, respectively [7-9]. Cells were cultured in monolayer in the above media supplemented with 10 % v/v fetal bovine serum (FBS) and 1 % v/v penicillin/ streptomycin (PS) cocktail at 37 ºC with 5 % CO_2 in humidified atmosphere. Media and sera were obtained from Nacalai Tesque and Thermo Fisher Scientific, respectively. It is noted that HSG is contaminated with human cervical carcinoma cell HeLa as shown by short tandem repeat analysis [10] but we used HSG in this study because it has long been used in earlier studies comparing the biological effects of various types of radiation including proton and heavy ion [11,12].

BPA which is enriched in ^{10}B (>98 %) was purchased from Katchem spol. s.r.o. (Prague, Czech Republic). For improved solubility, BPA-fructose complex was prepared as described before [13] and added to the cell culture media at indicated boron concentration. After keeping at 37 ºC for approximately 30 min to facilitate the uptake of BPA, cells were transported to the irradiation facility and waited for irradiation at the ambient temperature. Actual time between BPA administration and starting irradiation was 1 to 6.5 h in the case of HSG and 3 to 8 hr in the case of A375 and SAS.

2.2. Irradiation

UTR-KINKI was operated at 1 W output. Cells suspended in medium in 0.2 ml PCR tubes were placed at the center of the core in UTR-KINKI and irradiated at ambient temperature for 1 to 4 h, as indicated. The dose rates of neutron and γ-ray measured by paired chambers are reported to be 0.214 Gy h^{-1} and 0.196 Gy h^{-1}, respectively [2]. The flux of the neutron with the energy greater than a threshold of 0.3 MeV was reported to be 2.17×10^6 cm^{-2} s^{-1} [2]. The flux of thermal neutron measured in this study by Au foil activation was $(1.05 \pm 0.06) \times 10^7$ cm^{-2} s^{-1}.

2.3. Measurement of cell survival

Cell survival after irradiation was measured by colony formation assay. After irradiation, cells were diluted and plated onto 6 well plate so that 10-100 colonies are formed. Ten days to two weeks after plating, cells were fixed with either >99.5 % v/v ethanol or 4 % v/v formaldehyde and stained with 0.1 % w/v crystal violet solution and the number of colonies were counted. Plating efficiency (P.E.) was calculated as the number of colonies divided by the number of cells plated. P.E. of untreated control cells were 38.1±3.3 % for HSG, 103.6±4.6 % for A375 and 19.2±3.0% for SAS. Then, surviving fraction (S.F.) was obtained as the P.E. of irradiated cells divided by P.E. of unirradiated control cells. The cell survival curve was fitted to simple exponential function, *i.e.*, $\ln S.F. = -aT$, where T is irradiation time and "a" is a constant. Sensitivity enhancement ratio (SER) was obtained as the ratio of the "a" value with BPA to that without BPA.

3. Results and discussion

3.1. Cell killing effects of irradiation in UTR-KINKI

Irradiation in the nuclear reactor UTR-KINKI without BPA exerted substantial cell killing effects on HSG, A375 and SAS cells. Three hr irradiation decreased the surviving fraction of HSG, A375 and SAS to 0.24, 0.21 and 0.40, respectively (**Figure 1A-C**, black line and open circles). When the survival curves were fitted to the simple exponential function of $\ln S.F. = -aT$, the constant "a" was 0.42, 0.51 and 0.29 for HSG, A375 and SAS, respectively (**Table 1**).

In our recent study, we examined the effects of ^{137}Cs γ-ray irradiation on the survival of A375 and SAS. After 2 Gy γ-ray irradiation, S.F. of A375 and SAS was ~0.7 [7]. As the γ-ray dose in this study is estimated to be ~0.2 Gy h^{-1}, the cell killing effects of irradiation in UTR-KINKI are mostly attributable to neutron.

3.2. Enhancement of cell killing effects by BPA

BPA pretreatment significantly decreased the survival of HSG, A375 and SAS cells (Figure 1). There was the overall trend that the cell survival decreased in a manner dependent on BPA concentration (Figure 1), in agreement with a study by others [14]. It is noted, however, that the effects of 25 and 50 ppm boron concentration of BPA were similar in A375 cells. Although the exact reason for this apparent discrepancy is currently unclear, it may be underscored that the neutron dose and dose rate are low in the setting of this study and, thus, the neutron might have been mostly, if not fully, absorbed by lower concentration of BPA. The "a" value as well as α value in α-β model (linear quadratic model) might be expected to increase in a manner dependent on linear energy transfer. In

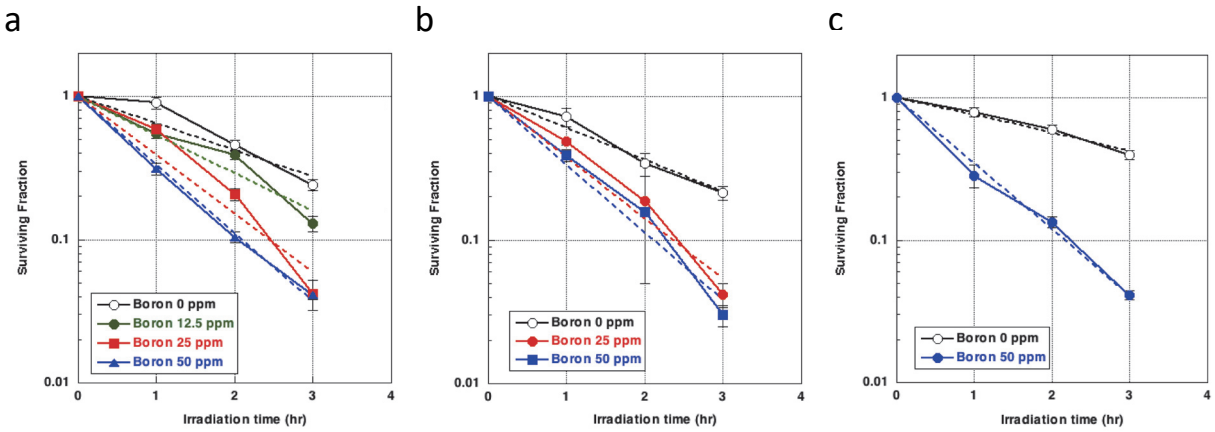

Figure 1. Surviving fraction of cultured human cancer cells after irradiation in UTR-KINKI with or without BPA. (a) salivary gland HSG cell, (b) melanoma A375 cell, (c) squamous cell carcinoma SAS cell. For each experimental group, cells were prepared and irradiated in triplicate. After irradiation, each replicate was plated in three to six plates for colony formation assay. The symbol and error bar represent the mean and standard deviation of the replicates, respectively. The dotted lines indicate the survival curves fitted to the function $\ln S.F. = -aT$.

Table 1. Cell killing and its enhancement by BPA of UTR-KINKI irradiation.

Cells	HSG				A375			SAS	
Boron concentration (ppm)	0	12.5	25	50	0	25	50	0	50
a	0.42	0.61	0.94	1.09	0.51	0.97	1.08	0.29	1.06
SER	-	1.45	2.23	2.58	-	1.91	2.13	-	3.71

agreement with this expectation, it was seen as a whole that "a" value increased in a manner dependent on BPA concentration, albeit that the effects of 25 and 50 ppm boron concentration of BPA were similar on HSG and A375 cells. SER of 50 ppm boron concentration of BPA was 2.58 for HSG and 2.13 for A375 but it was substantially higher, *i.e.*, 3.71, for SAS, which showed somewhat higher resistance to irradiation in the absence of BPA (Table 1).

In the above-mentioned study, we also measured the survival after irradiation in another nuclear reactor, *i.e.*, KURRI in Kyoto University, with 25 ppm boron concentration of BPA. After 1.2 Gy neutron irradiation, S.F. was ~0.1 for A375 and ~0.08 for SAS [7]. While the neutron dose in this study is estimated to be ~0.6 Gy after 3 hr irradiation, S.F. was <0.1 in both A375 and SAS. Thus, the cell survival after BNCT in this study appeared lower than that in our recent study.

4. Conclusion

The present study demonstrated the cell killing effects and its enhancement by neutron capture using BPA in UTR-KINKI of the output as small as 1 W. This reactor is shown potentially useful for the basic researches toward BNCT, such as the development of new boron-containing, neutron-capturing compounds and exploration for biomarkers.

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References

[1] S. Miyatake, M. Wanibuchi, N. Hu and K. Ono, Boron neutron capture therapy for malignant brain tumors, *Journal of Neuro-Oncology* 149 (2020), pp. 1-11.

[2] S. Endo, E. Yoshida, Y. Yoshitake, T. Horiguchi, W. Zhang, K. Fujikawa, M. Hoshi, T. Itoh, M. Ishikawa and K. Shizuma, Dosimetry of fission neutrons in a 1-W reactor, UTR-Kinki, *Journal of Radiation Research* 43 (2002), pp. 381-386.

[3] T. Ayaki, K. Fujikawa, H. Ryo, T. Itoh and S. Kondo, Induced rates of mitotic crossing over and possible mitotic gene conversion per wing anlage cell in *Drosophila melanogaster* by X rays and fission neutrons, *Genetics* 126 (1990), pp. 157-166.

[4] T. Itoh and S. Kondo, Somatic reversion of a Xantha-like gene in soybean by fast neutrons and X rays, *Japanese. Journal of Genetics* 66 (1991), pp. 461-469.

[5] K. Fujikawa, Y. Hasegawa, S. Matsuzawa, A. Fukunaga, T. Itoh and S. Kondo, Dose and dose-rate effects of X rays and fission neutrons on lymphocyte apoptosis in p53(+/+) and p53(–/–) mice, *Journal of*

Radiation Research 41 (2000), pp. 113-127.
[6] S. Adachi, H. Ryo, T. Hongyo, H. Nakajima, R. Tsuboi-Kikuya, Y. Tokita, F. Matsuzuka, K. Hiramatsu, K. Fujikawa, T. Itoh and T. Nomura, Effects of fission neutrons on human thyroid tissues maintained in SCID mice, *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 696 (2010), pp. 107-113.
[7] K. Kagawa, M. Murakami, Y. Hishikawa, M. Abe, T. Akagi, T. Yanou, G. Kagiya, Y. Furusawa, K. Ando, K. Nojima, M. Aoki and T. Kanai, Preclinical biological assessment of proton and carbon ion beams at Hyogo Ion Beam Medical Center, *International Journal of Radiation Oncology, Biology and Physics* 54 (2002), pp. 928-938.
[8] S. Imamichi, L. Chen, T. Ito, Y. Tong, T. Onodera, Y. Sasaki, S. Nakamura, P. Mauri, Y. Sanada, H. Igaki, Y. Murakami, M. Suzuki, J. Itami, S. Masunaga and M. Masutani, Extracellular Release of HMGB1 as an Early Potential Biomarker for the Therapeutic Response in a Xenograft Model of Boron Neutron Capture Therapy, *Biology* 11 (2022), p. 420.
[9] S. Masunaga, K. Ono, A. Takahashi, Y. Sakurai, K. Ohnishi, T. Kobayashi, Y. Kinashi, M. Takagaki and T. Ohnishi, Impact of the p53 Status of the Tumor Cells on the Effect of Reactor Neutron Beam Irradiation, with Emphasis on the Response of Intratumor Quiescent Cells, *Japanese Journal of Cancer Research* 93 (2005), pp. 1366-1377.
[10] L. Lin, O. Elkashty, M. Ramamoorthi, N. Trinh, Y. Liu, G. Sunavala-Dossabhoy, T. Pranzatelli, D.G. Michael, C. Chivasso, J. Perret, J.A. Chiorini, C. Delporte and S.D. Tran, Cross-contamination of the human salivary gland HSG cell line with HeLa cells: A STR analysis study, *Oral Disease* 24 (2018), pp. 1477-1483.
[11] M. Aoki-Nakano, Y. Furusawa, A. Uzawa, Y. Matsumoto, R. Hirayama, C. Tsuruoka, T. Ogino, T. Nishio, K. Kagawa, M. Murakami, G. Kagiya, K. Kume, M. Hatashita, S. Fukuda, K. Yamamoto, H. Fuji, S. Murayama, M. Hata, T. Sakae and H. Matsumoto, Relative biological effectiveness of therapeutic proton beams for HSG cells at Japanese proton therapy facilities, *Journal of Radiation Research* 55 (2014), pp.812-815.
[12] A. Uzawa, K. Ando, S. Koike, Y. Furusawa, Y. Matsumoto, N. Takai, R. Hirayama, M. Watanabe, M. Scholz, T. Elsässer and P. Peschke, Comparison of biological effectiveness of carbon-ion beams in Japan and Germany, *International Journal of Radiation Oncology, Biology and Physics* 73 (2009), pp. 1545-1551.
[13] A. Sato, T. Itoh, S. Imamichi, S. Kikuhara, H. Fujimori, T. Hirai, S. Saito, Y. Sakurai, T. Tanaka, H. Nakamura, M. Suzuki, Y. Murakami, D. Baiseitov, K. Berikkhanova, Z. Zhumadilov, Y. Imahori, J. Itami, K. Ono, S. Masunaga and M. Masutani, Proteomic analysis of cellular response induced by boron neutron capture reaction in human squamous cell carcinoma sas cells, *Applied Radiation and Isotopes* 106 (2015), pp. 213-219.
[14] I.H. Seo, J. Lee, D. Na, H. Kyung, J. Yang, S. Lee, S.J. Jeon, J.W. Choi, K.Y. Lee, J. Yi, J .Han, M. Yoo and S.H. Kim, The Anti-Tumor Effect of Boron Neutron Capture Therapy in Glioblastoma Subcutaneous Xenograft Model Using the Proton Linear Accelerator-Based BNCT System in Korea, *Life* 12 (2022), p.1264.