The biological effect of boron neutron capture reaction dose can be predicted by nucleo-cytoplasmic ratio or cell size

K. Ono[1], H. Tanaka[2], Y. Tamari[2], T. Watanabe[2], M. Suzuki[2], S.I. Masunaga[2].

[1]Osaka Medical College, Kansai BNCT Medical Center, Takatsuki, Japan.

[2]Kyoto University, Institute for Integrated Radiation and Nuclear Science, Kumatori, Japan.

Abstract

We re-analyzed the data of previously published papers to compare the differences in tumor cell radiosensitivity to ^{10}B $(n,\alpha)^{7}Li$ dose. Five tumors of EL4, SAS/neo, SAS/mp53, SCCVII and B16-BL6 melanoma were examined. In the combination of p-Boron-L-phenylalanine (BPA) and neutron beam, D_0 is the smallest for SAS/neo, and EL4, SAS/mp53, SCCVII and B16-BL6 melanoma increased in order. In the combined use of borocaptate disodium (BSH), D_0 of EL4 was the smallest, followed by SAS/neo, B16-BL6 melanoma, SAS/mp53 and SCCVII. The relationship between the nuclear-cytoplasmic ratio (Xnc) or cell size index (Xcs) and the D_0 value obtained by the pathology tissue slide image is: BPA-BNCT: $D_0=0.1341Xnc^{-1.586}$ ($R^2=0.9721$). In BSH-BNCT, $D_0=0.0122Xcs-0.1319$ ($R^2=0.9795$) for 4 tumors except B16-BL6 melanoma. Based on these results, we proposed a new biologically equivalent effect factor, absolute biological effect (ABE) ratio. The ABE factor is Gy/D_0. ABE dose is physical dose × ABE factor, which means the dose to reduce cell viability to $e^{-ABE\ dose/Gy}$

Introduction

Thermal neutron is captured by boron isotope ^{10}B nucleus at an incredibly high probability compared with those of other elements consisting the body and immediately splits the nucleus into α particle and Li nucleus: $^{10}B(n,\alpha)^{7}Li$. These particles have extremely short ranges that don't exceed the diameter of general cell and are high LET radiation with high RBE. Oxygen doesn't affect the cell killing effect of those particles. In clinical studies of BNCT using research reactors, excellent effects have already been reported in malignant brain tumor, malignant melanoma, head and neck cancer and so on. However, the relationship between the radiation dose given to the tumor and the effect is not necessarily. The simple sum of biologically equivalent dose obtained by multiplying each dose component by the fixed value of RBE or compound biological effectiveness (CBE) is taken as the total dose. Since the RBEs of two particles are extremely large, there may exist less possibility of the "variation" of the cell killing effect among tumors with different sensitivity to X-rays. However, we thought that it was necessary to confirm this point and reanalyzed our previously published data on experimental tumor models and searched for factors that determine the response of each tumor to $^{10}B(n,\alpha)^{7}Li$ dose.

Materials & Methods

Analysis of the relationship between boron-neutron dose and cell killing effect

n our previous papers used, SCCVII tumor, B16-BL6 melanoma, EL4 leukemia, human oral cancer SAS/neo and SAS/mp53, in which a mutant p53 gene was introduced into SAS/neo, in mice were irradiated by neutron beams in combination with BPA or BSH.The cell survival after BNCT were determined by colony formation assay.
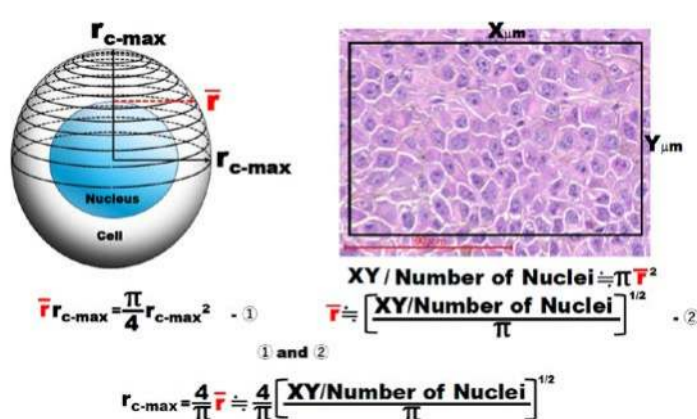
All neutron beam irradiations were performed by using the heavy water facility of KUR. Experiments of neutron beam alone without boron drugs were performed in every experiment, then the effect of boron-neutron dose to the tumor cell survival rate was obtained as the difference between slopes of the two exponentially decreasing straight lines.
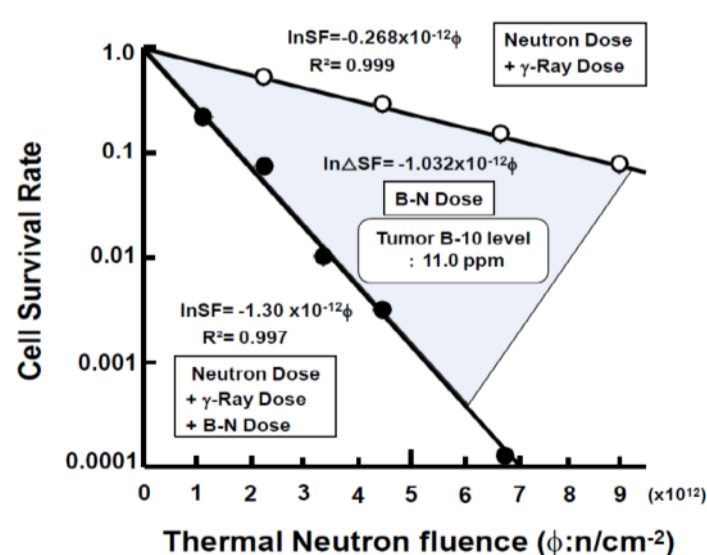
The neutron fluence necessary to lower the cell survival rate by e^{-1} on the subtracted line was defined as ϕ_0 similar to D_0 (Fig. 1). D_0 of boron-neutron dose was calculated based on ϕ_0 and the ^{10}B concentration in the tumor as follow: $D_0=\phi_0 \times 6.933 \times 10^{-14} \times ^{10}B$ concentration (ppm).

Search for nucleo-cytoplasmic (N/C ratio) and cell size index on the microscopic image of the histopathological slide

For the research, we used the Keyence BZ-8000 microscope with image analysis system built in. A region not containing the interstitial component as much as possible was selected on the image of HE staining histopathological slide and its area was measured. After that, using the image analysis software, the area occupied by the cytoplasm was determined by color tone and its area was also measured. The area obtained by subtracting the latter from the initial is the area occupied by the nuclei. The ratio of total area of the nuclear image to the initially selected area is the nucleo-cytoplasmic ratio. Similarly, when the number of nuclei in the selected area was counted and the area was divided by the number of nuclei, the value (μm²) becomes an indicator of the cell size.

Results

Table 1

Table 1 presents the data on D_0 of the boron-neutron dose in cell survival curve in which BPA or BSH were combined with neutron beam. The variation more than 2-fold was observed in both BPA-BNCT and BSH-BNCT depending on tumor type.

Tumor	$^{10}B(n,\alpha)^{7}Li$ Dose D_0(Gy)	
	BPA	BSH
EL4	0.43	0.75
B16-BL6	0.91	1.25
SAS/neo	0.35	1.2
SAS/mp53	0.52	1.44
SCCVII	0.74	1.6

Table 2

Table 2 shows the N/C ratio and cell size index of 5 tumors. EL4 had the smallest cell size index and the largest N/C ratio. In addition, the cell size of B16-BL6 melanoma was the largest, and the N/C ratio was the smallest.

Tumor	N/C volume ratio	Cell size index (μm²)
EL4	0.508	74.5
B16-BL6	0.3	202
SAS/neo	0.528	108
SAS/mp53	0.407	123
SCCVII	0.347	146

Figure 3 shows the relationship between the boron-neutron dose D_0 in BPA-BNCT and the N/C ratio (Xnc) of the cells. As the ratio increases, D_0 decreases. There is a very strong correlation between the two in the power approximation.
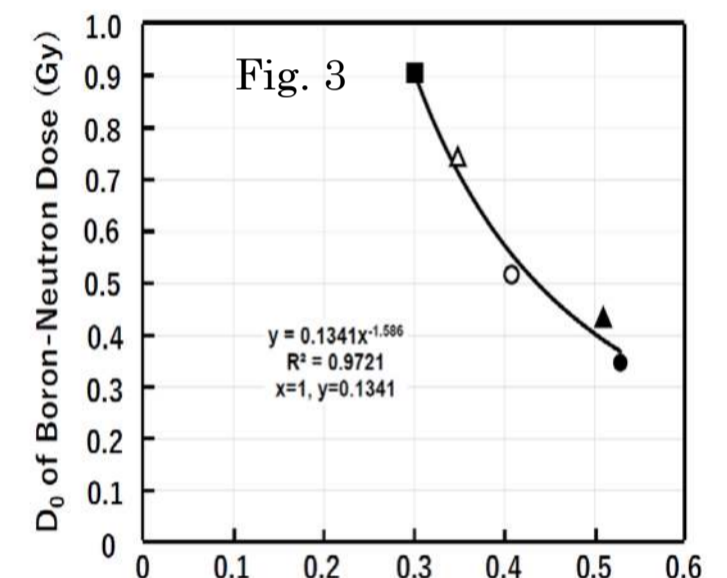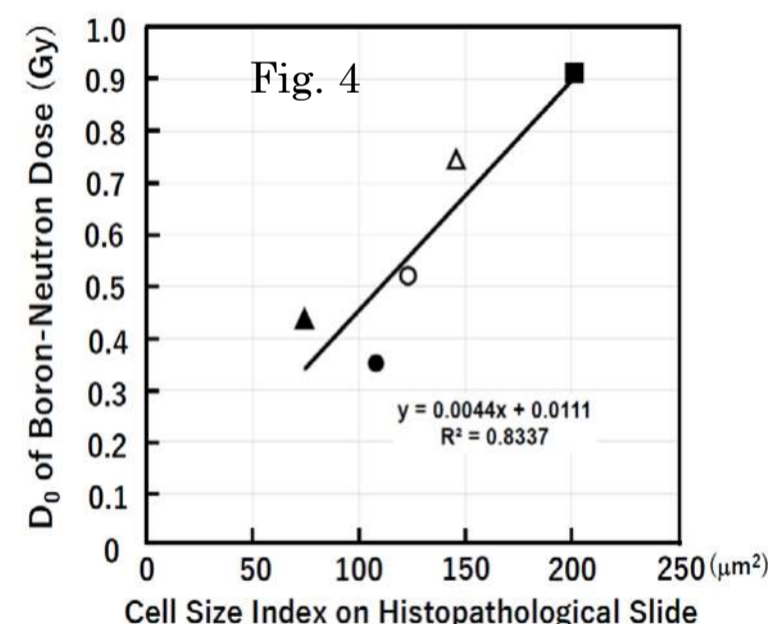

Figure 4 shows the relationship between the D_0 of the boron-neutron dose and cell size index in the BSH-BNCT. D_0 increased with increasing Xcs except for B16-BL6 melanoma. When restricted to four tumors, a very strong correlation is found in the linear approximation.

Proposal of new bioequivalent dose: absolute biologic effectiveness (ABE) dose

Since radio-sensitivity of tumor cells to boron-neutron dose in BNCT is uniquely determined, it is not necessary to use the coefficients RBE and CBE defined by the ratio to the radio-sensitivity to X-ray dose. As X-ray sensitivity of individual patient's tumor cells can't be determined, typical fixed values obtained by basic experiments are usually used. Therefore, RBE-Gy and CBE-Gy are never precise bioequivalent doses for individual tumors. It should be referred to as a convenient value.

Based on the results obtained in this study, we devised a new coefficient called absolute biologic effectiveness (ABE) factor. Its definition is ABE factor = Gy/ D_0. Since the unit of D_0 is Gy, the ABE factor is an anonymous number. ABE dose is obtained by multiplying this coefficient by the physical dose. This ABE dose means the dose that can reduce cell survival rate to $e^{-ABE\ dose/Gy}$. If the accumulated clinical data are analyzed with this bioequivalent dose, clonogenic cell number (stem cell number) in vivo may be estimated for each individual patient's tumor in the future.

For using ABE dose, it is necessary to precisely predict cell specific D_0. Therefore, it can be used only in BNCT which emits extremely high LET particles for the time being.