The RBE of Fractionated Fast Neutron on Walker 256 Carcinosarcoma with KCCH-Cyclotron

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For evaluation of biological effect of p^+(50.5 MeV) Be neutron beam produced by Korea Cancer Center Hospital (KCCH) cyclotron the RBE had been measured in experimental tumor Walker 256 carcinosarcoma as well as normal tissue, mouse intestine and bone marrow, in single and fractionated irradiation. As pilot study, the RBE had been measured for the mouse jejunal crypt cells in single whole body irradiation of which the result was 2.8. The obtained RBE values of TCD 50 of Walker 256 tumor, bone marrow and intestine in single irraiation were 1.9, 1.9 and 1.5 respectively. In fractionated irradiation, the RBE value of tumor Walker 256 was decreased as increasing of fraction number and increased as increaing of fraction size.

Key Words: RBE, Fast neutron, Walker 256 carcinosarcoma, KCCH-cyclotron

INTRODUCTION

The biologic effects of a radiation depend on the spatial distribution of the ionizing events deposited within the irradiated tissue. The rate at which charged particles deposit energy per unit distance is known as the linear energy transfer (LET)[1~3]. LET is the amount of energy transferred in unit distance of track which passed secondary recoil charged particles. The fast neutron is identified as high LET radiation because it reacts directly with nucleus of atom and is ionizing the tissue densely. RBE is the essential factor to define the biolgic effects in different quality of radiation. The standard radiation to compare biologic effect of neutron has been 250 KV X-ray, but recently it is accepted to use cobalt gamma ray as well. The factors affecting the RBE value are quality, radiation dose, dose rate, fractionation and biological end point. Moreover the major factor affecting the RBE is LET.

The KCCH-cyclotron accelerates proton in the energy of 50.5 MeV bombarding the beryllium target which is located in the neutron treatment gantry, Shown as p^+(50.5 MeV)Be reaction[4,5]. The authors measured RBE of neurtron beam produced from this accelerator for the biologic characterization for the fast neutron radiotherapy. The scope of this study covered measurement of neutron RBE in single and fractionated irradiation in various biological systems including normal tissue as well as experimental tumor Walker 256. The result obtained will be directly useful in clinical application of fast neutron beam of KCCH-cyclotron.

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MATERIALS AND METHOD

1. Fast Neutron Beam

Energy of neutron beam is 50.5 MeV peak and 24 MeV average. Depth of penetration is 14.5 cm of 50% depth dose level. Dmax is 13.5 mm. Width of penumbra is 8 mm in isodose curve between 80 to 20%. Treatment gantry is 360° full isocentric. The SAD of the machine is 150 cm. The depth dose distribution of beam is quite similar with the 6 MV X-ray(Fig. 1).

2. Objectives of Experiments

The main purpose of this study is measurement of RBE of an experimental tumor, Walker 256 carcinosarcoma, in single and fractionated irradiation and to define variance of RBE values in different biologic systems and different dose level. Reference radiation for neutron beam was Cobalt gamma rays. As pilot study, RBE measured in normal tissues of ICR mice. At first, RBE measured in mouse jejunal crypt cells in single irradiation that means standard normal tissue RBE. Next, RBE measured in mouse LD 50/6 as a representative of intestine and LD 50/30 as bone marrow in fractionated irradiation. And then, for main experiment, RBE mea-

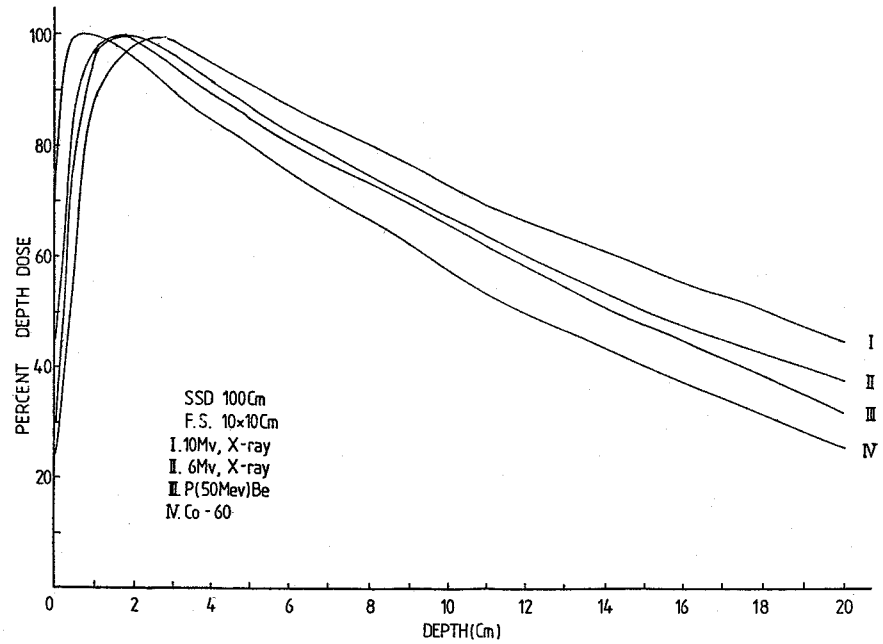

Fig. 1. Central aixs depth dose of various radiations measured at Korea Cancer Center Hospital.

sured in 50% tumor control dose, TCD50, of experimental tumor, Walker 256 carcinosarcoma, in fractionated irradiation.

3. The Tumor

The tumor, Walker 256 carcinosarcoma, was implanted to the Wistar rat onto the inguinal region using trocar needles, 2 to 3 fragments at a time, of 2 to 4 mm in size of each fragment. This tumor revealed no metastatic tendency, 100% growth after implant and no spontaneous regression during our experiment period. It grew 1 cm at one week after transplantation. For evaluation of tumor behavior, the authors observed growing pattern as a function of time (Fig. 2). Average diamter and average volume revealed typical sigmoid curve. It showed plateau of growth at 3 weeks after transplantation. For evaluation of radiation response of tumor, various doses of radiation had given and ovserved its response after irradiation (Fig. 3). The responders decreased its size immediately after irradiation and completely disappeared on 2 weeks after irradiation. Therefore, 50% tumor control dose, TCD50, was applied this two week responsiveness in calculation.

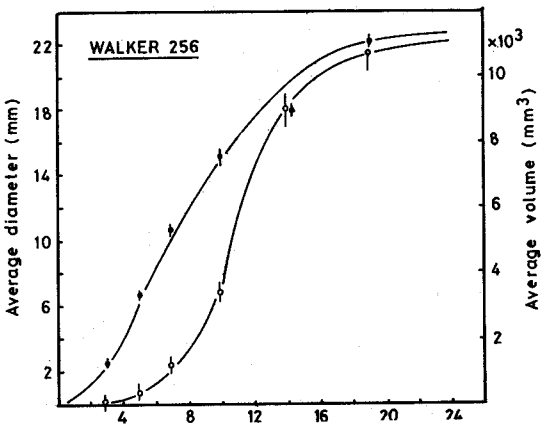

Fig. 2. Natural growth pattern of Walker 256 tumor after transplantation.

4. Irradiation and Evaluation

Cobalt treated group received irradiation by Cobalt therapy unit with 80 cm SSD and dose rate of 140 cGy/min. Neutron group received neutron irradiation with 150 cm SSD and dose rate of average 30 neutron rad/min. Dosimetry of neutron was

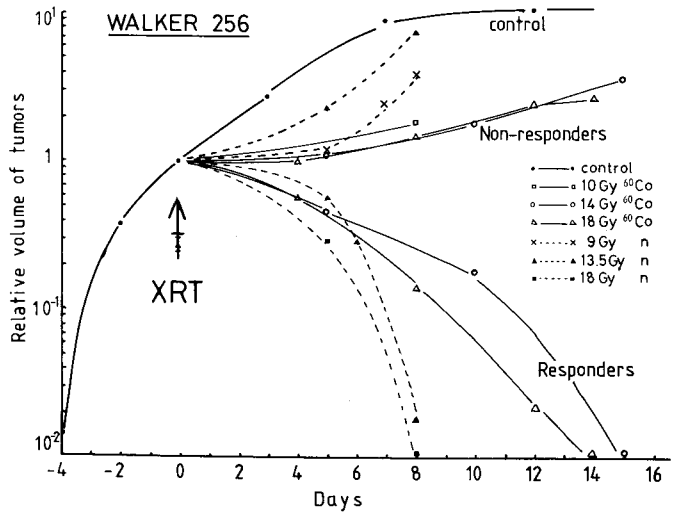

Fig. 3. Pattern of radiation response of Walker 256 tumor immediately after irradiation.

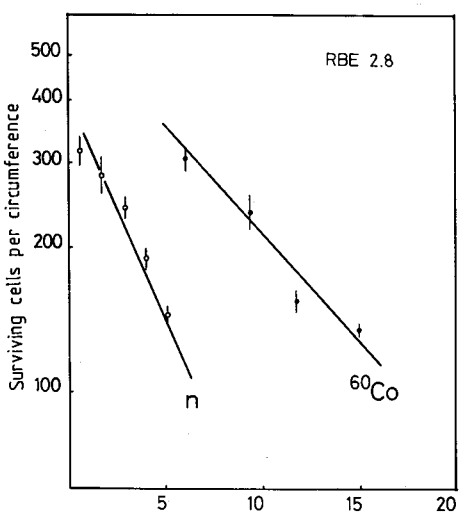

Fig. 4. Survival curve of mouse jejunal crypt cells in whole body single irradiation. Measured RBE was 2.8.

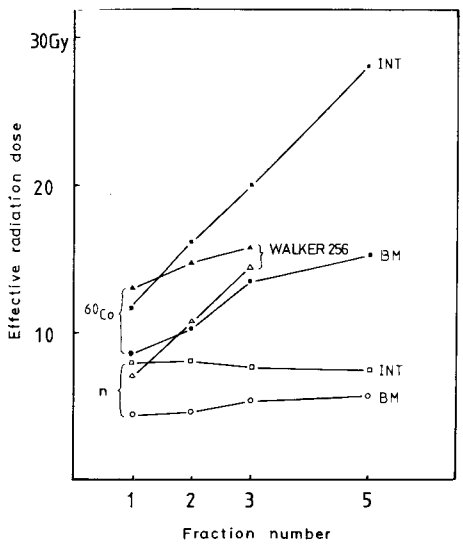

Fig. 5. The effective dose of various biological systems. (INT : Intestine, LD50/6, BM : Bone marrow, LD50/30, Walker 256 : TCD50). Changing pattern of effective dose as a function of fraction number is reversed in Walker 256 tumor compared to the normal tissue.

done with neutron ionization chamber (Far West Technology Inc.) and water phantom-electrometer system (Therados Co.).

For the jejunal crypt cell, single whole body irradiation was given to the four different dose groups of 6 mice in each group, then sacrifice and sampling of specimens of jejunum were carried out 80 hours after irradiation. Sampling of jejunal segment had taken out three separated pieces from the one cm in length of the intestine at the point of 2 cm distal to the end of the stomach. Count of number of regenerating jejunal crypt and calculation of jejunal crypt cells were carried out in usual manner adopted from Withers and Elkind method[6].

For the LD50/6 and LD 50/30, whole body irradiation was made in each four subgroups of different dose with 6 mice in each subgroup in single and fractionated irradiation as single fractions (1f/ld), 2 fractions in 2 days (2f/2d), 3 fraction in 3 days (3f/3d) and 5 fractions in 5 days (5f/5d) where total of 192 mice used. LD 50 had been calculated by the method of Dragstedt-Behrens[7].

For the tumor TCD50, local irradiation was made for the tumor grown in the inguinal region of the Wistar rat in each four subgroups of different dose with 6 rats (tumors) in single and fractionated irradiation as single fraction (1f/1d), 2 fractions in 2 days (2f/2d) and 3 fractions in 3 days (3f/3d), where total of 72 rats (tumors) used. Fifty percent tumor control dose had been calculated by the method of Dragstedt-Behrens under the daily observation and measurement of cross and longi-

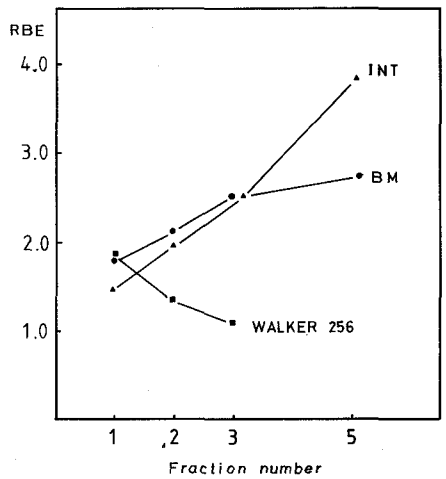

Fig. 6. RBE of each biological system. Increase of RBE as increase of fraction number is seen in normal tissue but RBE of Walker 256 tumor is reversed.

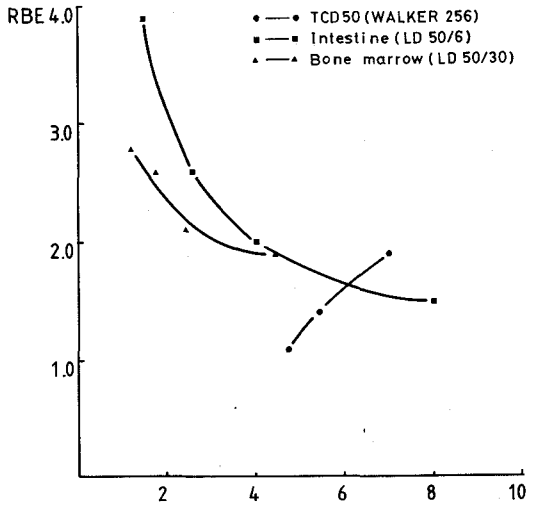

Fig. 7. RBE as a function of neutron dose in various biological systems. Decrease of RBE as increase of fraction size in normal tissue instead of increase in Walker 256 tumor.

Table 1. Dose Fractionation Studies for Each Biological Systems

Endpoint	Fractionation schedule	Dose (Gy)		RBE	Neutron dose (Gy/f)
		Neutron	Co-60 r-rays		
TCD 50 (Walker256)	1f/1d	7.00	13.05	1.9	7.00
	2f/2d	10.63	14.92	1.4	5.32
	3f/3d	14.54	16.00	1.1	4.85
LD 50/30	1f/1d	4.48	8.64	1.9	4.48
	2f/2d	4.75	10.20	2.1	2.38
	3f/5d	5.25	13.50	2.6	1.75
	5f/5d	5.48	15.12	2.8	1.10
LD 50/6	1f/1d	8.00	11.80	1.5	8.00
	2f/2d	8.08	16.00	2.0	4.04
	3f/5d	7.62	19.49	2.6	2.54
	5f/5d	7.19	28.00	3.9	1.44

Table 2. RBE Values Compared with Various Centers

Author	Mean energy beam	Gut		Marrow	
		RBE	Neutron dose (Gy)	RBE	Neutron dose (Gy)
Hornsey et al.	6 MeV	2.30	5.40	–	
Wambersie et al.	d-(33)-Be	2.40	4.05	–	
Broerse	15 MeV	1.40	9.48	1.10	6.59
Montour et al.	35 MeV	1.68	7.03	1.80	4.84
Muramatsu et al.	30 MeV	–		1.09	6.09
Geraci et al.	8 MeV	2.4	5.20	1.10	4.65
Radpath et al.	25 MeV	1.70	9.86	0.90	4.82
Gasinska et al.	5.6 MeV	2.25	3.77	1.84	3.38
Authors	p^+ (50.5 MeV) Be	1.5	8.00	1.9	4.48

tudinal diameter of tumor. Tumor control was determined disappearance of gross tumor two weeks after irradiation. There was no tumor which revealed regrowth until 5 weeks observation after disappearance.

RESULTS

Measured RBE of mouse jejunal crypt cells in single irradiation was 2.8 (Fig. 4).

Dose response of neutron and Cobalt gamma rays was determined in various biological systems of TCD 50 of Walker 256 tumor, LD 50/30 as a representative of bone marrow and LD 50/6 as intestine, in different fraction schedules (Table 1). RBE of Walker 256 tumor, mouse bone marrow and intestine in single irradiation were 1.9, 1.9, and 1.5 respectively. These values are changed in different fraction schedules. Dose response was also evaluated according to the neutron dose per fraction, i.e. fraction size. The variance of these results had been plotted in various graphs.

DISCUSSION

Determination of biologic characteristics of neutron beam is ultimately related to defining of the responses of normal and tumor tissues to the fast neutron beam. The possibility that cyclotron produced fast neutrons may enhance tumor control and cure rates in radiotherapy has resulted in the establishment of several research programs and clinical trials from the various centers in the world during last two decades[8,9]. Since the cyclotron at each of these centers is unique, the quality of the beams produced by these accelerators differs. Therefore, for the institutes who start first their neutron beam therapy, in devising the initial schedules and in understanding the consequences of modifying them, a great deal of work in experimental cellular and tissue radiobiology is needed[10]. Such experimental radiobiologic studies are also essential for the intercomparison of the effects of various neutron between the institutions and from other HIGH LET beams[11,12].

The method of biologic characterization in terms of RBE is described elsewhere[13~17]. The purpose of this study is upon the biological effectiveness of the fast neutron produced by the KCCH-cyclotron currently being used in radiotherapy at the Korea Cancer Center Hospital. The end points to be used in this study was mouse jejunal crypt cells, mouse intestine and bone marrow as normal tissue correspondence, and Walker256 carcinosarcoma as experimental tumor, in single and fractionated irradiation.

The rationale for using high energy neutrons for tumor therapy is based on their generally lower oxygen enhancement ratio (OER), less ability of repair from radiation damage (SLD & PLD), and minimum dependence of radiosensitivity of cell cycle, which in theory should reduce the disadvantages when the low LET radiation is employed[1,4,9]. Under conditions in which the radiation response of the tumor is governed mainly by these factors, use of neutrons might result in favorable therapeutic gain (RBE tumor/RBE normal tissue)[14]. Although this has not been consistently demonstrated because of the variation in RBE for various end-points and tissues, it is considered that in several animal tumor systems advantageous RBE ratios for tumor destruction compared to normal

tissue damage should have been found using neutrons.

Experimental tumor Walker 256 is a carcinosarcoma that arose spontaneously in the region of the mammary gland of a pregnant albino rat. This tumor was implanted onto the inguinal region of Wistar rat which grew well and revealed typical sigmoid curve during natural growth (Fig. 2). After irradiation of various radiation dose, they divided into two different groups: one of immediate decrease of size as responder and the other of growing continuously as non-responder. The responder revealed complete disappearance on two weeks later and none of them showed regrowth until 5 weeks observation after disappearance. Therefore the authors decided two week responsiveness to use in calculation of TCD 50 for the end-point(Fig. 3).

The technique for assaying survival of jejunal crypt stem cells for the evaluation of radiation response has been described elsewhere[6,10,18]. Cell survival curvce can be plotted using the number of crypts scored 3-2/3 days after the last irradiation by the method developed by Withers and Elkind[6]. The obtained RBE of mouse jejunal crypt cells in single irradiation of the presented study was 2.8 in exponential region (Fig. 4). Wihters et al reported 1. 33 in similar experiments and demonstrated no significant variability of these values in fractionated irradiations[10].

In evaluation of dose response of 50% death, LD 50, after whole body irradiation to the animals, LD 50/6 and LD 50/30 are widely used as the end-points. In mammals, death within one week of exposure to ionizing radiation is considered to be the result of damage to cells of the gastrointestinal epithelium (intestinal death). Death within one month is to be damage of bone marrow (bone marrow death) as well[9,14,19,20]. The radiation dose of 50% probability of death is easily calculated by the method of Dragstedt-Behrens[7].

Measurement of RBE of tumor in experiment is useful in comparison of RBE of tumor and normal tissues to determine the therapeutic ratio. Moreover, in fractionated irradiation, the result is valuable in consideration of neutron effect as a function of dose[14,21,22]. Neutrons are a more effective treatment than photons for those experimental tumors whose response is limited by hypoxic cells, because of low OER[22~24].

In the study of fractionated irradiation, the dose level must be taken into account for the determination of end-points. The RBE is higher in lower doses because repair from the sublethal damage takes place by photons which is expressed by the shoulder in the cell survival curve[1,20,21,25~27]. It is generally agreed in various investigators that the RBE values are decreased as the neutron dose per fraction is increased in normal tissues particularly in the gastrointestinal epithelium whose ability of repair from the sublethal damage is high[10,13,15,17,18~22,26,27].

The result of dose fractionation studies for each biological systems in the presented paper revealed that the obtained RBE values of TCD 50 of Walker 256 tumor, mouse LD 50/30 and LD 50/6 were 1.9, 1.9 and 1.5 respectively in single irradiation. In the evaluation of dose fractionation studies of neutron and photon, RBE values and neutron dose per fraction were changed according to the change of fractionation schedule (Table 1).

These results had been plotted in various graphs for intercomparison of radiobiologic responses of various end-points to the neutron and photon beam. The effective doses of normal tissue (Fig. 5), intestine and bone marrow, were markedly increased as increase of fraction number in cobalt treated mice instead of no change in neutron treated mice. However, response of Walker 256 tumor revealed less difference in cobalt treated group than neutron group.

RBE of normal tissue, intestine and bone marrow (Fig. 6), was considerably increased as increase of fraction number. However, RBE of Walker 256 tumor was decreased as increase of fraction number.

The obtained RBE as a function of neutron dose (Fig. 7) revealed considerable variety of each biologic systems. RBE of normal tissue, intestine and bone marrow, was decreased as increase of neutron dose per fraction. However, RBE of Walker 256 tumor was increased as increase of neutron dose per fraction.

These results has been compared with the results of other centers for the RBE of normal tissue, intestine and bone marrow (Table 2). The RBE values of presented study differ from those observed in the other centers but the range of all values were within 1.0 to 3.0.

CONCLUSION

By the experimental study to measure the RBE of tumor Walker 256 carcinosarcoma and to determine the variability of RBE values in fractionated fast neutron produced by the KCCH-cyclotron with the energy of p^+(50.5 MeV) Be as the biological

characterization prior to clinical application, the results obtained of RBE of Walker 256 carcinosarcoma were 1.9 in single irradiation.

In fractionated irradiation, this value was decreasd as increasing of fraction number and increased as increasing of fraction size.

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═국문초록═

Walker 256 Carcinosarcoma의 원자력병원 싸이클로트론 속중성자선 분할조사에 대한 생물학적 효과비에 관한 연구

한국에너지연구소 원자력병원 치료방사선과

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박 찬 일 • 강 위 생

원자력병원 싸이클로트론으로 발생되는 고에너지 속중성자선의 생물학적효과에 대한 연구의 일환으로 RBE를 측정하였다. 대상은 Walker 256 carcinosacoma 및 정상조직으로 마우스 위장관 및 골수를 이용하였고 단일 및 분할조사에 대한 반응을 관찰하였다. 마우스 공장소낭선세포의 단일전신조사에 의한 RBE는 2.8이었다. 단일조사시 이식암 Walker 256의 TCD 50에 대한 RBE, 마우스 골수 및 위장관의 RBE는 각각 1.9, 1.9, 1.5이었다. 분할조사시 Walker 256의 RBE는 분할횟수의 증가에 따라 감소하였고 1회 분할조사량의 증가에 따라 증가하였다.