

## Relative Biological Effectiveness of the 235 MeV Proton Beams at the National Cancer Center Hospital East

KOICHI ANDO<sup>1\*</sup>, YOSHIYA FURUSAWA<sup>1</sup>, MASAO SUZUKI<sup>1</sup>,  
KUMIE NOJIMA<sup>1</sup>, HIDEYUKI MAJIMA<sup>1</sup>, SACHIKO KOIKE<sup>1</sup>,  
MIZUHO AOKI<sup>1</sup>, WAKAKO SHIMIZU<sup>1</sup>,  
YASUYUKI FUTAMI<sup>2</sup>, TAKASHI OGINO<sup>3</sup>,  
SHIGEYUKI MURAYAMA<sup>3</sup>,  
and HIROSHI IKEDA<sup>3</sup>

<sup>1</sup>Clinical Radiation Biology and International Space Radiation Laboratory,

<sup>2</sup>Division of Accelerator Physics and Engineering, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan

<sup>3</sup>Department of Radiation Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa-shi, Chiba 277-8577, Japan

(Received, September 13, 2000)

(Revision received, December 25, 2000)

(Accepted, January 22, 2001)

### Relative biological effectiveness, human, mouse, cell survival, gut.

A therapy-dedicated cyclotron was installed in the National Cancer Center Hospital East (NCCHE) at Kashiwa in 1997. Prior to the start of clinical use, we investigated the biological effectiveness of therapeutic proton beams for cell lethality. The proton beams accelerated up to 235 MeV were horizontally extracted from the cyclotron, and scattered by a bar-ridge filter to produce a Spread-Out-Bragg-Peak (SOBP) of 10-cm width. The biological systems used here were mouse intestinal crypt cells and three in vitro cell lines, including SCC61 human squamous cell carcinoma, NB1RGB human fibroblasts and V79 Chinese hamster cells. The dose responses after irradiation at either the entrance plateau or the middle portion of SOBP were compared with those after linac 6 MV X-ray irradiation. The fit of a linear quadratic model to survival curves showed that proton irradiation increased the  $\alpha$  value of SCC61 and the  $\beta$  value of V79 cells with a least change for  $\alpha/\beta$  ratio of NB1RGB cells. The isoeffect dose that reduces either cell survivals to 10% or mouse jejunum crypts to 10 per circumference was termed  $D_{10}$ . The relative biological effectiveness (RBE) of protons obtained by comparing the  $D_{10}$  values between protons and X-rays ranged from 0.9 to 1.2. The depth distribution of cell lethality was measured by replating V79 cells after irradiation from a “cell stack chamber” that received a single dose of 7 Gy at the middle position of SOBP. The thus-obtained cell survivals at various depths coincided well with the estimated survivals, but tended to decrease at the distal end of SOBP. We conclude that an RBE of 1.1 would be appropriate for 235 MeV proton beams at the NCCHE.

\*Corresponding author: Phone; +81-43-251-2111 ext. 6925, +81-43-206-3231, Fax; +81-43-251-4531, E-mail; ando@nirs.go.jp

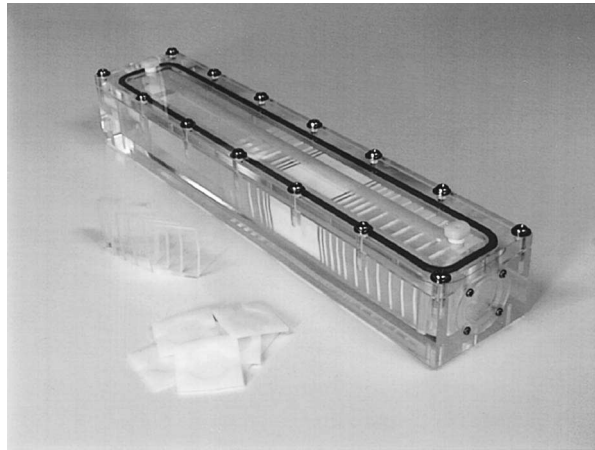
## INTRODUCTION

We previously compared the biological effectiveness of 70 MeV and 250 MeV proton beams, and obtained RBE values close to unity for both<sup>1)</sup>. Particle radiotherapy is currently attractive for oncologists as a new and promising modality to treat malignant tumors. The number of proton-therapy facilities in the world has increased in number from 5 to 16 within the last 10 years<sup>2)</sup>, and high tumor control rates for localized tumors, including retinal malignant melanomas, have been reported. In 1997, a therapy-dedicated cyclotron was installed in the NCCHE at Kashiwa<sup>3)</sup>. Between April and September of 1998, we investigated the biological effectiveness of therapeutic proton beams for cell lethality. Three cell lines, including human origin, were used for in vitro colony formation, while mouse gut crypt survivals provided in vivo response after a single dose of irradiation with 235 MeV proton beams. This energy of protons is used in clinical practice, because the beam intensity is sufficiently high for therapy, and because the depth doses in multiportal irradiation are quite achievable. We used linac 6 MV X rays as a reference beam to obtain proton RBE, which would provide more relevance to clinical practice than low-energy X rays or Co-60  $\gamma$  rays. We proposed an RBE of 1.1 for 235 MeV proton beams with 10-cm SOBP at the NCCHE.

## MATERIALS AND METHODS

### *Cell lines*

Two human cell lines, SCC61 and NB1RGB, and a Chinese hamster cell line V79 were used. Human SCC61 squamous cell carcinoma originated from the head and neck region<sup>4)</sup>. NB1RGB fibroblasts obtained from normal human skin<sup>5)</sup> were purchased from RIKEN cell bank (#RCB60222). The p53 status of the two human cell lines is wild type, while that of V79 is the mutated type. Exponentially growing cells were prepared as a monolayer in Eagle's medium (minimum essential MEM) supplemented with 10% fetal bovine serum (FBS). The incubator was kept at 37°C and 5% CO<sub>2</sub>. Cells were plated into 25 cm<sup>2</sup> plastic flasks (Nunc 152094), which were then filled up with a serum-supplemented medium. The plating efficiency of SCC61 cells was ~7% with feeder cells, while that of NB1RGB and of V79 was ~40% and ~80%, respectively (no feeder cells). All data collected from repeated experiments were combined. For depth distribution studies, V79 cells were plated on a round-shaped thermanox (Falcon) thin plate of 25 mm in diameter and 0.1 mm thickness. As many as 20 plates, 2 mm apart, were then transferred into a "cell stack chamber", a specially designed acrylate container with a serum-supplemented medium (Fig. 1). After irradiation, the plates were removed from the chamber to replat cells for colony formation. The cell stack method is superior to the gelatin method<sup>6)</sup>, such that the former requires a smaller number of cells to prepare and easier handling than the latter.



**Fig. 1.** Cell stack chamber and positioning plates.

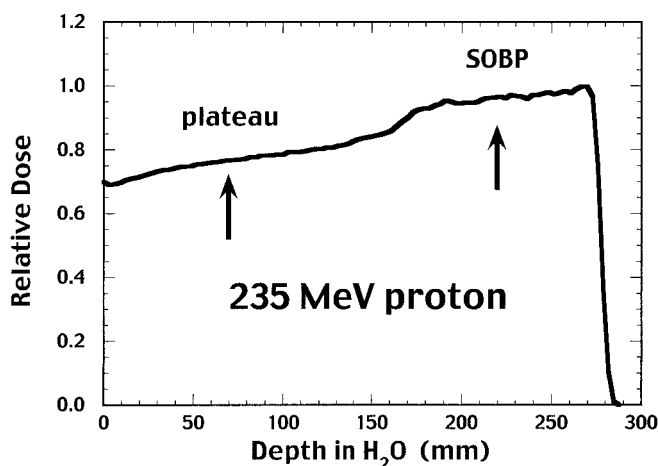
Horizontal proton beams enter from the right side of chamber. The depth position of V79 cells was adjusted by inserting an appropriate thickness of positioning plates (polyethylene and acrylate) between the beam entrance and the given position of the cells.

### *Animals*

C3H/HeMsNrsf female mice aged 12–18 week-old were used for this study. The animals were produced and maintained in specific pathogen-free (SPF) facilities at the National Institute of Radiological Sciences (NIRS). The mice were transported to the accelerator facility 2 to 4 days before irradiation. All data collected from repeated experiments were combined. A total of 114 mice were used for the experiments, with 3 mice for each irradiation-dose point.

### *Irradiation*

Horizontal proton beams were accelerated up to 235 MeV by the cyclotron at the NCCHE. The Bragg peak at 272 mm depth in water equivalence was spread out by a bar ridge filter, producing an SOBP of 10 cm width (Fig. 2). Proton beams perpendicularly spread by a double-scattering method<sup>7)</sup> were shaped down by a brass collimator to form a field size of 11 cm × 11 cm. This provided a field of 97 mm in diameter with a homogeneity of 2.5% dose difference. Dosimetry was conducted using JARP Farmer type dosimeter<sup>8,9)</sup>. The cell stack chamber was irradiated with protons such that a dose of 7 Gy was delivered to 220 mm depth in water equivalence. For dose-response studies, the irradiation depth was adjusted by placing polyethylene plates of appropriate thickness at a position between the collimator and the samples. Mouse gut and cells were irradiated at either the entrance plateau of 77 mm- or the SOBP of 220 mm-depth in water equivalence. The dose rate ranged from 1.6 to 2.7 Gy/min. Mice were intraperitoneally injected with pentobarbital anesthesia of 50 mg/kg. Being taped down on an acrylate plate, the mice received to the abdomen a single dose with either proton or X rays in the ventral-to-dorsal direction. Reference beams of 6 MV X rays were generated by a linac therapy machine at the NCCHE. A polyethylene block of 50 mm thickness was placed at a position between the collimator and the samples. The lateral dose distribution



**Fig. 2.** Spread-Out-Bragg-Peak (SOBP) of 235 MeV protons. The cells and intestine were irradiated at the entrance plateau (left arrow) and middle of the SOBP (right arrow).

within a 2.5% dose difference was provided by the collimator for a field size of 10 cm  $\times$  10 cm. The dose rate was 2.7 Gy/min. Irradiation was carried out at room temperature, and the experiments were repeated twice.

#### *in vitro cell survival assay*

Cells were brought back to the NIRS immediately after irradiation. NB1RGB and V79 cells were replated into 60 mm plastic dishes for survival assays. SCC61 cells were, without replating, returned to the incubator. After 7- and 14-day incubation at 37°C for V79 and human cells, respectively, colonies were stained with a solution of either methylene blue or crystal violet. The number of colonies per dish was counted, and the surviving fractions were calculated as the ratio of the plating efficiencies for irradiated and unirradiated cells.

#### *in vivo crypt survivals*

Irradiated mice were kept alive in animal facilities at the NCCHE for 3.5 days after irradiation. Jejunum was removed and cut into 10 pieces with 3 mm length each. Fixed in a 10% neutral formalin for several days, jejunum samples were processed to a histology preparation of H & E staining. The number of crypts per transverse circumference was counted microscopically, and averaged to plot on a semilogarithmic scale against the dose. We previously used this crypt survival assay for RBE studies of carbon ions<sup>10</sup>.

#### *Survival parameters and RBE*

*In vitro* dose-cell survivals were fitted to the linear quadratic model. A multiple regression analysis was used to calculate the means and standard deviations for the  $\alpha$  and  $\beta$  values of each cell line. The dose required for reducing surviving fraction to 10% was calculated by using these  $\alpha$  and  $\beta$  values (here termed  $D_{10/\text{cell}}$ ). The crypt survivals were fitted to an expo-

nential function. The mean and standard deviations were obtained for each  $D_0$  value. The dose required for reducing the number of crypts per circumference down to 10 was calculated (here termed  $D_{10/\text{crypt}}$ ). The RBE was calculated by comparing the  $D_{10}$  values of proton beams with those of 6 MV X rays (designated as  $\text{RBE}_{(D_{10})}$ ).

## RESULTS

### *Cell and crypt survivals*

The dose-response curves for in vitro cell lines are shown in Fig. 3. SCC61 cells possessed curvilinear survival curves, and were moderately radiosensitive (Fig. 3 a). The SOBP proton killed SCC61 cells slightly more efficiently than the linac X rays, while the plateau proton showed a survival curve indistinguishable from the linac X rays. Human fibroblasts NB1RGB showed a trivial bend on the survival curve of linac X rays (Fig. 3 b). The dose responses after proton irradiation were similar between the SOBP and the plateau, and were marginally steeper than those after experiencing linac X rays. V79 Chinese hamster cells were most radioresistant among the three cell lines used here, exhibiting a distinct bend with a pronounced curvature (Fig. 3 c). The surviving fractions after SOBP proton irradiation were lower than those after experiencing linac X rays for all dose points. The plateau proton showed a survival curve similar to that of the linac X rays. The crypt survivals in logarithm were well fit to linear regression against the dose in normal scale (Fig. 3 d). The SOBP proton reduced crypt cells as efficiently as the linac X rays, while the plateau proton was least effective.

The survival parameters for linac X rays and protons are listed in Table 1. For SCC61 cells, the  $\alpha$  values tended to be larger than that of the linac X rays, while the  $\beta$  values remained stable. The ratio of  $\alpha/\beta$  was  $1.460 \text{ Gy}^{-1}$  for X rays, and increased to  $5.532 \text{ Gy}^{-1}$  for the SOBP proton, even though the increase was statistically insignificant. The  $\alpha/\beta$  ratios of NB1RGB cells were constant, irrespective of the radiation qualities. Both the  $\alpha$  and  $\beta$  values of any protons were marginally larger than those of linac X rays. V79 cells possessed an  $\alpha/\beta$  ratio similar to that of NB1RGB cells after linac X-ray irradiation. The plateau proton produced a smaller  $\alpha$ , but a larger  $\beta$ , than the linac X rays, resulting in a smaller  $\alpha/\beta$  ratio of  $6.977 \text{ Gy}^{-1}$ . The SOBP proton showed an  $\alpha$  value similar to that of linac X rays. The  $D_0$  values of gut crypt cells ranged from 1.32 Gy and 1.41 Gy, and were similar between protons and linac X rays.

### *RBE*

The  $D_{10}$  and  $\text{RBE}_{(D_{10})}$  values are listed in Table 2. SCC61 showed a  $D_{10/\text{cell}}$  of 3.87 Gy for linac X rays, and presented  $\text{RBE}_{(D_{10})}$  values of 1.0 and 1.2 for the plateau proton and SOBP, respectively. Protons at any position required a slightly smaller  $D_{10/\text{cell}}$  than did linac X rays for NB1RGB fibroblasts, and showed a common RBE value of 1.1. V79 cells possessed the largest  $D_{10/\text{cell}}$  after linac X rays among the three cell lines. The  $D_{10/\text{cell}}$  after experiencing plateau protons was similar to that after experiencing X rays, while the  $D_{10/\text{cell}}$  after experiencing

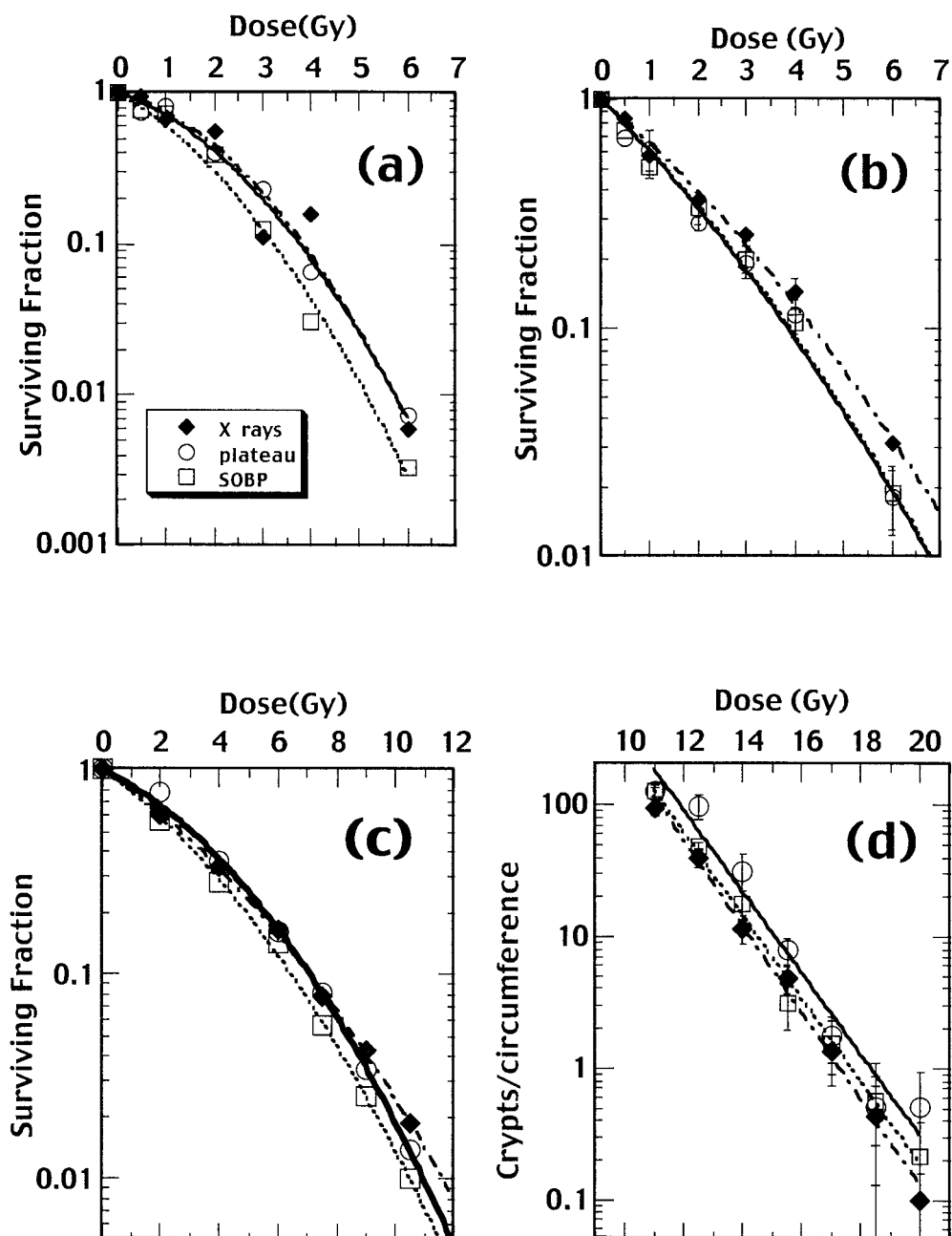


Fig. 3. Dose-response curves. A linear quadratic model is fit to SCC61 (a), NB1RGB (b), and V79 (c) while exponential regression is fit to jejenum crypts (d). The symbols and bars are the mean and sd obtained from 3 samples.  $\blacklozenge$ , Linac X rays;  $\circ$ , plateau proton;  $\square$ , SOBP proton.

**Table 1.** Survival Parameters

			$\alpha$ (Gy <sup>-1</sup> )	$\beta$ (Gy <sup>-2</sup> )	$\alpha/\beta$ (Gy)
SCC61	X rays		$(1.623 \pm 2.399) \times 10^{-1}$	$(1.112 \pm 0.392) \times 10^{-1}$	$1.460 \pm 2.219$
	Proton	Plateau	$(2.453 \pm 0.789) \times 10^{-1}$	$(0.967 \pm 0.129) \times 10^{-1}$	$2.536 \pm 0.883$
		SOBP	$(4.726 \pm 1.140) \times 10^{-1}$	$(0.854 \pm 0.186) \times 10^{-1}$	$5.532 \pm 1.780$
NB1RGB	X rays		$(3.515 \pm 0.521) \times 10^{-1}$	$(3.475 \pm 0.851) \times 10^{-2}$	$10.116 \pm 2.896$
	Proton	Plateau	$(4.006 \pm 0.713) \times 10^{-1}$	$(4.009 \pm 1.166) \times 10^{-2}$	$9.993 \pm 3.407$
		SOBP	$(3.919 \pm 0.495) \times 10^{-1}$	$(4.096 \pm 0.810) \times 10^{-2}$	$9.567 \pm 2.246$
V79	X rays		$(2.095 \pm 0.160) \times 10^{-1}$	$(1.604 \pm 0.147) \times 10^{-2}$	$13.062 \pm 1.558$
	Proton	Plateau	$(1.657 \pm 0.220) \times 10^{-1}$	$(2.375 \pm 0.202) \times 10^{-2}$	$6.977 \pm 1.101$
		SOBP	$(2.137 \pm 0.205) \times 10^{-1}$	$(2.126 \pm 0.188) \times 10^{-2}$	$10.049 \pm 1.312$
D0 (Gy)					
gut crypt	X rays		$1.324 \pm 0.04$		
	Proton	Plateau	$1.407 \pm 0.122$		
		SOBP	$1.377 \pm 0.056$		

a: mean and sd

**Table 2.** D<sub>10</sub> and RBE

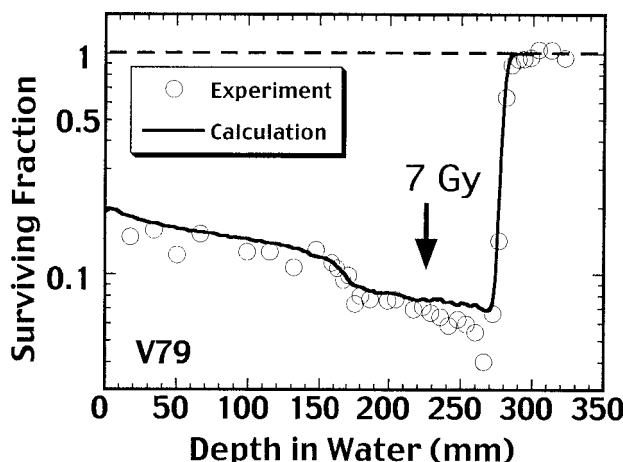
	6MV X rays	235 MeV Proton			
		Plateau		Spread-Out-Bragg-Peak	
		D <sub>10/cell</sub> or D <sub>10/crypt</sub> (Gy)	D <sub>10/cell</sub> or D <sub>10/crypt</sub> (Gy)	RBE	D <sub>10/cell</sub> or D <sub>10/rypt</sub> (Gy)
human squamous cell carcinoma <sup>1)</sup>	3.87 ± 3.09 <sup>3)</sup>	3.77 ± 1.18	1.03 ± 0.88	3.11 ± 2.09	1.24 ± 1.30
human fibroblast <sup>2)</sup>	4.52 ± 0.0	4.08 ± 0.83	1.11 ± 0.23	4.11 ± 0.51	1.10 ± 0.14
hamster V79 cell	7.11 ± 0.98	6.95 ± 1.23	1.02 ± 0.23	6.53 ± 1.22	1.09 ± 0.25
mouse jejunum crypt	14.22 ± 0.20	15.10 ± 2.08	0.94 ± 0.13	14.49 ± 1.41	0.98 ± 0.10

<sup>1)</sup> SCC61, <sup>2)</sup> NB1RGB, <sup>3)</sup> mean and sd

SOBP protons was marginally small. In gut crypt survivals, linac X rays of 14.22 Gy were required for D<sub>10/crypt</sub>, while a D<sub>10/crypt</sub> of 15.10 Gy was for plateau protons. The proton SOBP showed a D<sub>10/crypt</sub> similar to that of linac X rays, and resulted in an RBE value of unity.

#### Depth distribution of effectiveness

The cell lethality along the proton beam path was investigated. The relationship between the surviving fraction and the depth is shown in Fig. 4. The surviving fraction after 7 Gy was 0.071 for the cell stack chamber (223 mm depth), similar to 0.0074, which was obtained for the culture bottle (220 mm depth; Fig. 3c). The surviving fraction was 0.147 at the entrance (18 mm depth in water equivalence), and gradually decreased with depth. The surviving frac-



**Fig. 4.** Survivals of V79 cells irradiated with proton beams at various depth positions. A physical dose of 7 Gy at the position indicated by the arrow was delivered to a cell stack chamber. Each symbol is the mean of 3 plates, to which cells at given position were replated after irradiation.

tions varied from 0.07 and 0.08 at the proximal SOBP, but decreased with depth at the distal SOBP to below 0.06. Reaching a minimum level of 0.04 at 266 mm depth, the surviving fraction suddenly increased to 0.89 at 285 mm depth. The product of two factors, i.e., the physical dose and the surviving fraction relative to 220 mm depth, was calculated and plotted as an estimated survival in Fig. 4. The estimation is based on the assumption that survivals after a dose of  $d$  at any depth are given by

$$SF = \exp(-0.214 \cdot d - 0.0213 \cdot d^2),$$

where the two constants,  $\alpha$  and  $\beta$ , are those experimentally obtained for the 220 mm depth (Fig. 3c). The estimated survivals well fit to the experimental survivals, including the fall-off edge of beams. The distal end of SOBP showed a tendency that the experimental survivals were lower than the estimated values.

## DISCUSSION

We have reported here the RBE values of 235 MeV proton beams accelerated by a cyclotron at the NCCHE at Kashiwa. The employed reference beams were 6 MV X rays, which have been clinically used in the hospital. The biological effectiveness of 6 MV X rays was smaller by 10% than that of 200 kVp X rays for our cell lines (unpublished data). The RBE of particle beams generally depends on several factors, including LET, dose per fraction and biological system. In radiotherapy, the RBE for fast neutrons varies from 2 to 5, while that for protons ranges within smaller limits, i.e., 1.0 to 1.2<sup>11)</sup>. The present results show that a RBE<sub>(D10)</sub>



of 235 MeV proton beams ranged between 1.0 and 1.2 for in vitro cell kill, and coincided with other reports<sup>12)</sup>. The  $\text{RBE}_{(\text{D}_{10})}$  of protons for gut crypt cells in the present study was 1.0 or smaller. This does not support an opinion that the gastrointestinal tissues may be relatively more sensitive to protons<sup>12)</sup>.

The RBE of protons could depend on fraction size. Using  $\alpha$  and  $\beta$  values in the present studies, the isoeffect doses of protons that match  $\text{SF}_2$  (surviving fraction produced by 2 Gy of X rays) were calculated to obtain the RBE values at  $\text{SF}_2$ , i.e.,  $\text{RBE}_{(\text{SF}_2)}$ . The  $\text{RBE}_{(\text{SF}_2)}$  values of the plateau protons for NB1RGB and V79 were 1.1 and 0.9, respectively, while those of the SOBP protons for NB1RGB and V79 were 1.1 and 1.1, respectively. These RBE values at the  $\text{SF}_2$  were similar to those obtained at  $\text{D}_{10}$  in the present study. However, the  $\text{RBE}_{(\text{SF}_2)}$  values of plateau- and SOBP protons for SCC61 were 1.1 and 1.5, respectively, and slightly larger than the  $\text{RBE}_{(\text{D}_{10})}$  values. SCC61 cells after X ray irradiation showed smaller  $\alpha$ - and  $\alpha/\beta$  values than the other two cell lines. The high LET component of the distal SOBP protons (see discussion in the next paragraph) could be important for cells with small  $\alpha$ - and  $\alpha/\beta$  values. The RBE values for late complications of normal tissues, which also show small  $\alpha/\beta$  values, could be large after distal SOBP proton irradiation. The RBE of protons does not depend on the dose for mammalian tissues<sup>13)</sup>, even though the alpha/beta ratios may affect the proton RBE for cultured cells<sup>14)</sup>. The RBE values obtained for 235 MeV proton beams at the NCCHE appears to be identical to those obtained for other clinically-used protons with energy ranging from 70 to 200 MeV<sup>12,15,16)</sup>. Accordingly, an RBE value of 1.1, which has been used at other institutes, could be applied to high-energy protons, such as 235 MeV. Protons with a small RBE contrast with carbon ions with large RBE values<sup>17,18)</sup>.

The depth distribution of biological effectiveness using the cell stack chamber showed that the distal end of 10-cm SOBP was more effective than the proximal half of SOBP (Fig.4). In the present study, the RBE tended to depend on the depth, such that the SOBP position showed larger RBE values than the plateau for all biological systems, except for NB1RGB fibroblasts (Table 2). The RBE dependence on depth may be caused by a high LET component at the distal end of the SOBP proton<sup>19)</sup>. The RBE dependence on depth is reported for protons with low energy (65–70 MeV)<sup>20, 21)</sup>, but not clear for high-energy 200 MeV protons<sup>22)</sup>.

In conclusions, we propose a RBE of 1.1 for a 235 MeV proton with 10-cm SOBP at the NCCHE. Further studies are warranted to investigate the biological effectiveness in a SOBP narrower than 10 cm, where the RBE values could be greater than the 1.1 obtained in the present study.

## ACKNOWLEDGEMENTS

We thank Miss.Chisa Oohira, Miss.Chizuru Yamaguchi, Miss.Lee Ryonfa for their technical assistance. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan and by the funds for Research Project with Heavy Ions at the National Institute of Radiological Sciences - Heavy-ion Medical Accelerator in Chiba (NIRS-HIMAC).

## REFERENCES

1. Ando, K., Koike, S., Kawachi, K., Hiraoka, T., Ohara, H., Yokota, M., Inada, T., Hirokawa, Y., Sato, S., Eguchi, K. and Urano, M. (1985) Relative biological effectiveness of the therapeutic proton beams at NIRS and Tsukuba University. *NIPPON ACTA RADIOL.* **45**: 531–535.
2. Sisterson, J. M. (1998) World wide proton therapy experience in 1997. *Jpn. Soc. Ther. Radiol. Oncol.* **9**, Suppl. 2: 46.
3. Ogino, T. (1998) Science and ethics of proton therapy. *Japanese Journal of Cancer & Chemotherapy* **25** (2): 274–281.
4. Nagasawa, H., Keng, P., Maki, C., Yu, Y., Little, J. B. (1998) Absence of a radiation- induced first-cycle G1-S arrest in p53+ human tumor cells synchronized by mitotic selection. *Cancer Research* **58** (9): 2036–2041.
5. Tsuboi, K., Tsuchida, Y., Nose, T., Ando, K. (1998) Cytotoxic effect of accelerated carbon beams on glioblastoma cell lines with p53 mutation: clonogenic survival and cell-cycle analysis. *International Journal of Radiation Biology* **74** (1): 71–79.
6. Blakely, E. A. (1995) Biological beam characterization. In: *Ion beams in tumor therapy*. Ed. Ute Linz, pp.63–72, Chapman & Hall, London.
7. Gottschalk, B. (1986) Capability of passive beam spreading techniques. *Proc. 5th PTCOG Meeting and International Workshop on Biomedical Accelerators*. pp.161–168.
8. Vynckier, S., Bonnett, D. E., Johns, D. T. (1991) Code of practice for clinical proton dosimetry. *Radiotherapy & Oncology* **20**: 53–63.
9. Vynckier, S., Bonnett, D. E., Johns, D. T. (1994) Supplement to the code of practice for clinical proton dosimetry. *Radiotherapy & Oncology* **32**: 174–179.
10. Fukutsu, K., Kanai, T., Furusawa, Y., Ando, K. (1997) Response of mouse intestine after single and fractionated irradiation with accelerated carbon ions with a spread-out Bragg peak. *Radiation Research* **148** (2): 168–174.
11. Wambersie, A. (1999) RBE, reference RBE and clinical RBE: applications of these concepts in hadron therapy. *Strahlentherapie und Onkologie* **175** Suppl 2: 39–43.
12. Raju, M. R. (1995) Proton radiobiology, radiosurgery and radiotherapy. *International Journal of Radiation Biology* **67** (3): 237–259.
13. Urano, M., Verhey, L. J., Goitein, M., Tepper, J. E., Suit, H. D., Mendiondo, O., Gragoudas, E. S., Koehler, A. (1984) Relative biological effectiveness of modulated proton beams in various murine tissues. *International Journal of Radiation Oncology, Biology, Physics* **10** (4): 509–514.
14. Gerweck, L. E., Kozin, S. V. (1999) Relative biological effectiveness of proton beams in clinical therapy. *Radiotherapy & Oncology* **50** (2): 135–142.
15. Munzenrider, J. E., Austin-Seymour, M., Blitzer, P. J., Gentry, R., Goitein, M., Gragoudas, E. S., Johnson, K., Koehler, A. M., McNulty, P., Moulton, G., et al. (1985) Proton therapy at Harvard. *Strahlentherapie* **161** (12): 756–763.
16. Habrand, J. L., Haie-Meder, C., Rey, A., Mammar, H., Pontvert, D., Gaboriaud, G., Couanet, D., Lenir, C., Valinta, D., Ferrand, R., Boissarie, G., Beaudre, A., Kerody, K., Mazal, A., Dupouy, N., Bonomi, M., Mazon, J. J. (1999) Radiotherapy using a combination of photons and protons for locally aggressive intracranial tumors. Preliminary results of protocol CPO 94-C1. *Cancer Radiotherapie* **3** (6): 480–488.
17. Han, Z. B., Suzuki, H., Suzuki, F., Suzuki, M., Furusawa, Y., Kato, T., Ikenaga, M. (1998) Relative biological effectiveness of accelerated heavy ions for induction of morphological transformation in Syrian hamster embryo cells. *Journal of Radiation Research* **39** (3): 193–201.
18. Ofuchi, T., Suzuki, M., Kase, Y., Ando, K., Isono, K., Ochiai, T. (1999) Chromosome breakage and cell lethality in human hepatoma cells irradiated with X rays and carbon-ion beams. *Journal of Radiation Research* **40** (2): 125–133.
19. Coutrakon, G., Cortese, J., Ghebremedhin, A., Hubbard, J., Johanning, J., Koss, P., Maudsley, G., Slater, C. R., Zuccarelli, C. (1997) Microdosimetry spectra of the Loma Linda proton beam and relative biological effective-

- ness comparisons. *Medical Physics* **24** (9): 1499–1506.
20. Courdi, A., Brassart, N., Herault, J., Chauvel, P. (1994) The depth-dependent radiation response of human melanoma cells exposed to 65 MeV protons. *British Journal of Radiology* **67**: 800–804.
  21. Wouters, B. G., Lam, G. K., Oelfke, U., Gardey, K., Durand, R. E., Skarsgard, L. D. (1996) Measurements of relative biological effectiveness of the 70 MeV proton beam at TRIUMF using Chinese hamster V79 cells and the high-precision cell sorter assay. *Radiation Research* **146** (2): 159–170.
  22. Gueulette, J., Bohm, L., De Coster, B. M., Vynckier, S., Octave-Prignot, M., Schreuder, A. N., Symons, J. E., Jones, D. T., Wambersie, A., Scalliet, P. (1997) RBE variation as a function of depth in the 200-MeV proton beam produced at the National Accelerator Centre in Faure (South Africa). *Radiotherapy & Oncology* **42** (3): 303–309.