Biological Intercomparison Using Gut Crypt Survivals for Proton and Carbon-Ion Beams

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RBE/Spread-out Bragg peak/Charged particle therapy.

Charged particle therapy depends on biological information for the dose prescription. Relative biological effectiveness or RBE for this requirement could basically be provided by experimental data. As RBE values of protons and carbon ions depend on several factors such as cell/tissue type, biological endpoint, dose and fractionation schedule, a single RBE value could not deal with all different radiosensitivities. However, any biological model with accurate reproducibility is useful for comparing biological effectiveness between different facilities. We used mouse gut crypt survivals as endpoint, and compared the cell killing efficiency of proton beams at three Japanese facilities. Three Linac X-ray machines with 4 and 6 MeV were used as reference beams, and there was only a small variation (coefficient of variance < 2%) in biological effectiveness among them. The RBE values of protons relative to Linac X-rays ranged from 1.0 to 1.11 at the middle of a 6-cm SOBP (spread-out Bragg peak) and from 0.96 to 1.01 at the entrance plateau. The coefficient of variance for protons ranged between 4.0 and 5.1%. The biological comparison of carbon ions showed fairly good agreement in that the difference in biological effectiveness between NIRS/HIMAC and GSI/SIS was 1% for three positions within the 6-cm SOBP. The coefficient of variance was < 1.7, < 0.6 and < 1.6% for proximal, middle and distal SOBP, respectively. We conclude that the inter-institutional variation of biological effectiveness is smaller for carbon ions than protons, and that beam-spreading methods of carbon ions do not critically influence gut crypt survival.

INTRODUCTION

The number of particle therapy facilities in Japan has increased from 1 to 10 in the past 15 years. Carbon-ion therapy at NIRS (National Institute of Radiological Sciences), Chiba had treated more than 2800 patients by the year 2006,1) whereas more than 250 patients have been treated with carbon ions at GSI(Gesellschaft für Schwerionenforschung mbH), Darmstadt, Germany since 1997.2) As the biological effectiveness of particle beams for therapy is important in terms of determining the dose prescription, we have already experimentally evaluated the RBE (relative biological effectiveness) values of proton beams at 2 Japanese facilities.3,4) Using mouse gut crypt survival as an endpoint, we have obtained and here report the RBE values of protons at 3 facilities that recently started operation in Japan. As to carbon-ion beams, we also have been comparing the biological effectiveness of the spread-out Bragg peak between Chiba/HIMAC and GSI/SIS synchrotrons. Biological comparison for carbon ions showed fairly good agreement between Chiba and GSI under an identical SOBP profile.

METHODS AND MATERIALS

Animals

C3H/He female mice 10 to 12 weeks old were used. Mice used for two proton facilities at Wakasa and at Shizuoka were purchased commercially, while mice used at other facilities were produced in our institute. Mice were transported to each facility 2 or 3 days before irradiation. Anesthesia with ketamine and xyladine was used for carbon-ion irradiation because of legal regulations for animal experimen-
Irradiation

Proton beams with 180, 190 and 200 MeV were accelerated by synchrotrons and used for experiments at Wakasa Wan Energy Research Center, Shizuoka Cancer Center, and University of Tsukuba, respectively. The abdomen of mice was irradiated with single doses of proton beams, the Bragg peak of which were spread out to 6-cm width. Two positions within the beam path were used, the entrance plateau and the middle of the spread-out Bragg peak.

Carbon ions were accelerated to 290 MeV/u by HIMAC and SIS synchrotrons, and spread out to 6-cm width. The SOBP profile for the SIS synchrotron used in the experiment was adjusted to be the same as the SOBP profile being used for therapy at Darmstadt. The mouse jejunum was placed at 3 positions within the SOBP, i.e., middle, 2-cm upstream and 2-cm downstream of the SOBP.

Reference X-rays used for proton RBE studies were obtained by Linac machines with 4 MV (at University of Fukui) and 6 MV (at Shizuoka Cancer Center and at National Cancer Center Hospital East).

Endpoint

Crypt survivals were histologically measured. The jejunums of mice were removed and fixed in formalin 3.5 days after irradiation. Histology preparations were made, and H&E staining was used to count microscopically the number of crypts surviving and regenerating. For non-irradiated control, the number of crypts per circumference section was between 120 and 145. Experiments were repeated 2 or 3 times for each proton facility, and the data obtained from each were combined for use. X-ray data obtained by 3 Linac machines were also combined for use.

The RBE values of protons were calculated by comparing the iso-effect doses obtained on survival curves between protons and X-rays. Obtained on survival curves were 3 iso-effect doses of $D_{30}$, $D_{10}$ and $D_{3}$, the doses required to reduce survivals to 30, 10, and to 3 crypts, respectively.

RESULTS

Proton RBE

Crypt survival curves after reference Linac X-ray irradiation are shown in Fig. 1. As the 3 Linac X-rays produced similar dose-crypt survivals (Fig. 1A), we combined all data (Fig. 1B) to use as a reference for proton RBE studies. The iso-effect doses to reduce crypt survivals to 30, 10 and 3, i.e., $D_{30}$, $D_{10}$ and $D_{3}$, respectively, were calculated and listed in Table 1. Coefficient of variance (C.V.) was 1.7–2.0%.

Figure 2 shows the crypt survival curves obtained at 3 pro-

Table 1. Iso-effect doses of reference X-rays. $D_{30}$, $D_{10}$ and $D_{3}$ values are calculated from crypt survival curves shown in Fig. 1.

| X-ray energy (MV) | Facilities | $D_{30}$ (Gy) | $D_{10}$ (Gy) | $D_{3}$ (Gy) |
|------------------|------------|--------------|--------------|--------------|
| 4                | Fukui*1    | 12.32        | 14.09        | 16.03        |
| 6                | Shizuoka*2 | 12.29        | 13.82        | 15.49        |
| 6                | Kashiwa*3  | 12.74        | 14.29        | 15.98        |

Mean and SD for $D_{30}$, $D_{10}$ and $D_{3}$ are 12.45 ± 0.25, 14.07 ± 0.24 and 15.83 ± 0.3, respectively.

*1: University of Fukui, *2: Shizuoka Cancer Center *3: National Cancer Center Hospital East.
ton facilities. Cell kill by entrance plateau was less effective than that by SOBP in all 3 facilities. For Wakasa protons, SOBP was more effective than X-rays, while the entrance resulted in a survival curve similar to X-rays. The entrance of Shizuoka protons also showed a survival curve similar to X-rays, whereas SOBP was again more effective than X-rays. For Tsukuba protons, SOBP was least effective and was similar to X-rays. The entrance of Tsukuba protons was less effective than X-rays.

From the data in Fig. 2, we calculated the iso-effect doses for proton beams (Table 2). X-ray iso-effect doses were calculated from the data in Fig. 1B. The coefficient of variance for entrance and SOBP ranged between 4.0 and 5.1%.

RBE values of protons were calculated from the iso-effect doses and listed in Table 3. Mean values were obtained by averaging 3 RBE values for each irradiation position. Proton beams at Wakasa showed an averaged RBE value of 1.01 and 1.10 at entrance plateau and SOBP position, respectively. Proton beams at Tsukuba showed smaller RBE values than those of Wakasa, namely, 0.93 for entrance and 1.00 for

| Table 2. Iso-effect doses of protons. | Facilities | Beam Position | D30 (Gy) | D10 (Gy) | D3 (Gy) |
|-------------------------------------|------------|---------------|----------|----------|---------|
| Wakasa*1                            | Entrance   | 12.36         | 13.93    | 15.65    |
|                                     | SOBP       | 11.39         | 12.77    | 14.28    |
| Shizuoka*2                          | Entrance   | 12.56         | 14.05    | 15.69    |
|                                     | SOBP       | 12.25         | 13.60    | 15.07    |
| Tsukuba*3                           | Entrance   | 13.58         | 15.11    | 16.79    |
|                                     | SOBP       | 12.55         | 14.07    | 15.74    |

*1: Wakasa Wan Energy Research Center, *2: Shizuoka Cancer Center, *3: University of Tsukuba.

Table 3. RBE values of protons. Iso-effect doses of protons listed in Table 2 were compared with mean values of X-ray doses shown as marginal notes of Table 1.

| Facilities | Beam Position | D30 | D10 | D3   | mean and SD |
|------------|---------------|-----|-----|------|--------------|
| Wakasa     | Entrance      | 1.01| 1.01| 1.01 | 1.01 ± 0.00  |
|            | SOBP          | 1.10| 1.10| 1.11 | 1.10 ± 0.00  |
| Shizuoka   | Entrance      | 1.00| 1.00| 1.01 | 1.00 ± 0.01  |
|            | SOBP          | 1.02| 1.04| 1.05 | 1.04 ± 0.01  |
| Tsukuba    | Entrance      | 0.92| 0.93| 0.94 | 0.93 ± 0.01  |
|            | SOBP          | 1.00| 1.00| 1.00 | 1.00 ± 0.00  |

Fig. 2. Crypt survivals after proton irradiation. Gut was irradiated with entrance plateau (circle) and middle SOBP (square) of proton beams at Wakasa Wan Energy Research Center (A), Shizuoka Cancer Center (B) and the University of Tsukuba (C). The solid line for X-rays is identical to that in Fig. 1 B.

Fig. 3. Depth-dose distributions of 290 MeV/u carbon ions at Chiba and Darmstadt. Gut was irradiated with 6-cm SOBP at three positions shown as arrows. Black line is for GSI/SIS synchrotron while red line is for NIRS/HIMAC synchrotron.
SOBP. Proton beams at Shizuoka showed intermediate values of RBE. In all 3 facilities, RBE of the entrance position was smaller than that of the SOBP position.

**Carbon ion studies**

Figure 3 shows the beam profiles of carbon ions used in the experiments at GSI and NIRS. Depth-dose distribution was identical between the two carbon beams. Mouse jejunum was irradiated at 3 positions within SOBP.

Experiments were repeated 2 or 3 times, and the data obtained were combined to construct crypt survival curves at each position as shown in Fig. 4. Fairly good agreement between HIMAC and SIS was observed. The iso-effect doses are listed in Table 4. Coefficient of variance was 0.7–1.7, 0.3–0.6 and 0–1.6% for proximal, middle and distal SOBP, respectively.

Dose ratios of HIMAC over SIS are listed in Table 5. The difference in biological effectiveness between NIRS/HIMAC and GSI/SIS was 1% for the three positions within the 6-cm SOBP.

**DISCUSSION**

Inter-comparison of therapeutic particle beams using identical endpoints has been thoroughly done for fast neutrons, but scarcely for protons. Gueulette et al. used the same crypt survival model to compare the biological effectiveness of protons among 5 institutes.10 RBE values ranged from 1.07 to 1.18 among the facilities, and they were determined by comparing the iso-effect doses for 20 crypts (D20) between protons at the middle of 7-cm SOBP and cobalt γ rays. In the present study, we compared the biological effectiveness of therapeutic protons at 3 facilities, obtaining proton RBE values ranging from 0.92 to 1.01 for the entrance plateau and from 1.0 to 1.11 for the middle of 6-cm SOBP. Applying the same endpoint as used in this study, we previously studied and reported proton RBE values for the National Cancer Center Hospital East and Hyogo Ion Beam Medical Center as 0.94 and 1.01 at the entrance plateau, and as 0.98 and 1.05 at the middle SOBP, respectively. Two values of proton RBE for the National Cancer Center Hospital East reported by Gueulette et al.10 and Ando et al.3 are slightly different (1.08 vs. 0.94), even though the identical strain of C3H mice produced in our institute was used. Gen-

**Table 4.** Iso-effect doses of carbon ions. D30, D10 and D3 values are calculated from crypt survival curves shown in Fig. 4.

| Facilities | Beam Position in SOBP | D30 (Gy) | D10 (Gy) | D3 (Gy) |
|------------|-----------------------|----------|----------|---------|
| GSI        | Proximal              | 9.23     | 10.21    | 11.51   |
|            | Middle                | 8.19     | 9.40     | 10.72   |
|            | Distal                | 7.25     | 8.37     | 9.61    |
| NIRS       | Proximal              | 9.24     | 10.38    | 11.62   |
|            | Middle                | 8.23     | 9.46     | 10.81   |
|            | Distal                | 7.08     | 8.29     | 9.61    |

**Table 5.** Dose ratio of HIMAC and SIS for D30, D10 and D3

| Beam Position in SOBP | D30 | D10 | D3 | Mean and SD |
|-----------------------|-----|-----|----|-------------|
| Proximal              | 1.02±0.01 | 1.02 | 1.01 | 1.02 ± 0.01 |
| Middle                | 1.00 | 1.01 | 1.01 | 1.01 ± 0.00 |
| Distal                | 0.98 | 0.99 | 1.00 | 0.99 ± 0.01 |

*1: (an iso-effect dose for NIRS-HIMAC) / (an iso-effect dose for GSI-SIS).
erally observed in any facility was that the RBE value at middle SOBP was slightly larger than the RBE value at entrance plateau. This observation agrees with other reports stating that proton RBE increases along with the beam path.\textsuperscript{11)}

It is generally accepted that proton RBE for therapeutic beams is 1.1.\textsuperscript{12)} This value is close to the maximum RBE obtained in the present report. What is notable in the present results is that the RBE of proton SOBP was close to 1.0 at Tsukuba, lower than that of 1.11 at Wakasa. The small RBE values of 1.0 or less that have been obtained for Tsukuba (Table 3) is puzzling. As experiments using \textit{in vitro} colony formation assay also show small RBE values for Tsukuba (data not shown), the proton beams at Tsukuba would possess any unique characteristics in physics. As the biological factors possibly affecting RBE values such as endpoint, mouse strain, and dose range/size were unified in this intercomparison, the \textasciitilde 10\% difference in RBE between facilities would be due to certain factors related to physics. Further studies of biology and physics are required to clarify the difference of RBE between facilities. We should also doubt whether the biological effectiveness was the same between cobalt $\gamma$ rays and Linac X-rays. Figure 5 illustrates and compares the published data of crypt survivals that were obtained by irradiation with 235 MeV protons at the National Cancer Center Hospital East, Kashiwa, Japan. Mice used in the 2 experiments were both C3H strain, and they were produced at NIRS. The two survival curves in the left panel are for SOBP with 7-cm width\textsuperscript{10)} and 6-cm width,\textsuperscript{3)} and are almost identical. However, the right panel shows that the reference radiation of cobalt $\gamma$ rays resulted in survival curves apparently different from Linac X-rays. This difference between reference radiations resulted in different RBE values, \textit{i.e.}, 0.98 with Linac X-rays\textsuperscript{3)} and 1.08 with cobalt $\gamma$ rays,\textsuperscript{10)} even though the biological effectiveness of proton beams in the two experiments was same.

The biological effectiveness of carbon ions was almost identical between NIRS and GSI (Fig. 4, Table 5). This also means that the difference in beam modulation methods between NIRS and GSI,\textsuperscript{8,13)} \textit{i.e.}, scatterer vs. raster scan, is not critical to the homogeneously irradiated gut, a visceral organ. Spot-scanning proton beams at the Paul Scherrer Institute (PSI)\textsuperscript{13)} produces large variations in local dose deposition to mouse gut, reportedly due to intestinal movement during irradiation.\textsuperscript{14)} In our experiments, HIMAC carbon ions are perpendicularly spread by use of a scatterer and wobblers, and not spot-scanned.\textsuperscript{10)} GSI carbon ions are spread by use of the raster scan method, which is similar to the spot scan method.\textsuperscript{15)} The PSI proton experiment used 10,000 spots for a 1-liter volume,\textsuperscript{14)} while \textasciitilde 80,000 spots were used in our GSI experiments. Also, we used anesthesia that may have reduced intestinal movement during irradiation, even though such kind of medical action is not well known.

**CONCLUSIONS**

We conclude that the biology of particle beams is not only important for evaluating the RBE values but is also essential for standardizing particle therapy throughout the world. Direct comparison of biological effectiveness between Linac X-rays and cobalt-60 gamma rays is particularly important for proton therapy, and would be reported by us in near future.

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