Particle biology

Evaluation of therapeutic gain for fractionated carbon-ion radiotherapy using the tumor growth delay and crypt survival assays

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ABSTRACT

Background and purpose: The aim of the study was to evaluate the therapeutic gain of carbon ion (C-ion) radiotherapy using a mouse model.

Materials and methods: Transplanted fibrosarcoma (NFSa) growing in C3H/He mice and murine small intestine were irradiated with 290 MeV/nucleon C-ion beams (C-ions) in 1–12 fractions separated by 4 h. The cell killing efficiencies of C-ions were measured using jejunum crypt survival and tumor growth delay (TGD) assays.

Results: The equieffect dose for crypt survival and TGD increased with increasing number of fractions after X-rays and 20 keV/μm C-ions, whereas TGD after 77 keV/μm C-ions rather decreased. Crypts showed stronger LET-dependent increase in α terms than the tumor while β terms less depended on LET irrespective of tissues. Therapeutic gain factor, i.e., a ratio of tumor RBE over crypt RBE, of 77 keV/μm C-ions was more than unity at any doses while that of 20 keV/μm C-ions increased with an increase in dose per fraction.

Conclusions: These specific data imply that use of large dose per fraction would be suitable for C-ion radiotherapy irrespective of LET from the point of view of therapeutic gain, though small dose per fraction by high-LET radiation decreases total dose for tumor.

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Carbon-ion beams (C-ions) are known to have a superior dose distribution associated with the sharp penumbra and the Bragg peak, allowing for highly conformal irradiation of deep seated tumors, together with a higher biological effectiveness than X-rays [1,2]. Several clinical trials have been completed or are currently underway to evaluate the clinical role of C-ions [3–5]. Fractionated irradiation is a valuable tool in conventional radiotherapy (RT) to reduce early and late effects in normal tissue, by allowing repair of sublethal damage or reoxygenation of a hypoxic tumor. In carbon-ion (C-ion) RT, superiority of the physical dose distribution leads to a reduction in the number of fractions [6] or may even allow hypofractionation. Experiments involving fast neutron beams have demonstrated that increasing the dose per fraction tended to lower the relative biological effectiveness (RBE) regarding both tumor and normal tissues [7]. However, the dose-dependent decrease in the RBE for the tumor is less pronounced than that for normal tissues such as skin and lung [8]. These experiments led to the assumption that the therapeutic gain of C-ion RT would increase when the fraction dose increased. This assumption has been confirmed in animal experiments that have compared RBE between tumor and skin [9,10]. As the intestine is an organ at risk in RT for deep-seated tumor adjacent to the gastrointestinal tract, it is important to investigate how fractionation influences the therapeutic gain of C-ion RT for normal intestinal tissues and tumors. In the present study, we evaluated the therapeutic gain of C-ion fractionation using crypt survival and tumor growth delay (TGD) assays to obtain a biological basis for the establishment of the optimal fraction strategy in C-ion RT.

Materials and methods

Mice and tumor

The animals were bred and maintained in the specific pathogen free conditions. C3H/He male mice aged 8 weeks were used for the tumor study. Syngeneic NFSa fibrosarcoma cells of the 16th through

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18th generations were transplanted intramuscularly into the right hind legs of mice at 10 days before the first irradiation. For the crypt experiment, C3H/He female mice aged 10–12 weeks were used. Four mice were used for each irradiation dose point. The animals involved in these studies were procured, maintained and used in accordance with the Recommendations for Handling of Laboratory Animals for Biomedical Research, compiled by the Committee on the Safety and Handling Regulations for Laboratory Animal Experiments of NIRS, and by the guidelines of the Animal Care and Experimentation Committee of Gunma University, Showa Campus.

Irradiation

C-ions were accelerated to 290 MeV/u using the HIMAC synchrotron and spread out to a width of 6 cm. The desired linear energy transfer (LET) was obtained by inserting a given thickness of polymethyl methacrylate upstream of the mice. C-ions with a dose-averaged LET of 20 keV/μm were obtained at the entrance of the plateau, while those with a dose-averaged LET of 77 keV/μm were located within the spread-out Bragg peak. A desired irradiation field was obtained by the simultaneous use of an iron collimator and a brass collimator. X-rays with a dose rate of 1.3 Gy/min were used as the reference beam for determining the RBE. The dose-averaged LET for 200 kV X-rays is 9.4 keV/μm [11]. Fractionation involved equal radiation doses given at 4-h intervals. The dose-responses regarding crypt survival were obtained for 1, 2, 4, 6, 8, 10 and 12 fractions, while those regarding TGD were obtained for 1, 2, 3, 4, 5, 6, 8, 10 and 12 fractions. Equal dose per fraction was used in all experiments. For the tumor study, four mice were immobilized on a Lucite plate to locate their right hind legs in a 28 × 100 mm rectangular field, and they received either a single dose or fractionated doses at 4-h intervals. The foot was excluded from the irradiation field. For the crypt study, mice were immobilized on a Lucite jig that had been especially designed for gut irradiation [12], and were irradiated with horizontal beams. The jejunum was surgically removed from the mice at 3.5 days after single-dose irradiation. For fractionation, the jejunum was removed from mice at 3.5 days after the half of the total doses were given.

Endpoints and data analysis

Measurement of tumor volume was used as the TGD assay. The tumor volume was calculated according to the following formula: (length × width × height × π)/6, and was plotted against time (days) after irradiation. The tumor growth (TG) time, that is the time required for each tumor to increase by five times its initial volume, was calculated from the first day of irradiation, and the TG times obtained for all animals were averaged for each dose group. The difference between the TG time of an experimental group and that of a non-irradiated control was defined as the TGD time. The specific TGD (sTGD) time was defined as the TGD time divided by the TG time of the non-irradiated control. The crypt survival assay was performed as described previously [12].

To analyze the effectiveness of various fractionation schemes, a dose–response curve was constructed by plotting the sTGD time. This dose response curve was used to obtain an equieffect dose; this was defined as the radiation dose necessary to produce either a sTGD time of 2.0 or a crypt survival of 10.0. The data for each dose response curve were fitted to a quadratic polynomial function using the least-squares method. The 95% confidence limit around the equieffect dose was calculated using the Maharanobis distance [13].

The RBE value (mean ± 95% confidence limit) was obtained using the following formula:

$$\text{RBE}\,(X/C) = (X/C) \pm (X/C) \times \sqrt{\{(x/X)^2 + (c/C)^2\}},$$

where X and C are the mean equieffect doses in a given fractionation regimen by X-rays and C-ions, respectively, and x and c are the 95% confidence limits for X-rays and C-ions, respectively.

Comparing the RBE for the TGD to the RBE for the crypt survival, we calculated therapeutic gain factor (TGF) of C-ions to evaluate significance of dose- and/or fraction sizes in C-ion RT. The TGF is different from therapeutic ratio (TR) that is widely used for various remedies for diseases, even though both indicate benefits of modality when values are larger than unity. The TGF was obtained using the following formula:

TGF = (the RBE value for the TGD)/(the RBE value for the crypt survival)

Statistical comparisons were performed using the regression analysis or the unpaired *t*-test. $p < 0.05$ represented statistical significance.

Results

Dose–response of the crypt survival and NFSa fibrosarcoma

Figs. 1 and 2 show the crypt survival and sTGD for each fractionation regimen plotted against the total dose of X-rays, 20 keV/μm C-ions or 77 keV/μm C-ions, respectively. As the LET increased, the dose–response curves for both crypt survival and the sTGD shifted to the left. The dose–response curves for both the crypt survival and the sTGD for X-rays and 20 keV/μm C-ions shifted to the right when the number of fractions increased, while the shift was not apparent for 77 keV/μm C-ions. The dose response curves of crypt survival for single dose irradiation were almost identical in comparing X-rays with 20 keV/μm C-ions, while 20 keV/μm C-ions showed stronger effects than X-rays for fractionated irradiation. The dose response curves of the sTGD for 20 keV/μm C-ions moved upward and separated from those of X-rays at high doses, even though these curves overlapped each other at low doses.

We calculated the equieffect doses for the crypt survival and the sTGD from the dose-response curves (Fig. 3). The equieffect dose for crypt survival slightly increased with an increase in the number of fractions for X-rays and 20 keV/μm C-ions. No fractionation effects were observed for 77 keV/μm C-ions. The equieffect dose for the sTGD progressively increased with an increase in the number of fractions for X-rays until the increase became less pronounced when the number of fractions exceeded six. The equieffect dose of the sTGD for 20 keV/μm C-ions progressively increased with the increase in the number of fractions, while that for 77 keV/μm C-ions significantly ($p = 0.0007$) decreased with an increase in the number of fractions.

LET dependence of the α and β terms

Using Fe-plots, we analyzed the equieffect dose to evaluate the dependence of the α and of the β terms on LET (Fig. 4). The α and β terms contain an equieffect surviving fraction (E) that produces a given magnitude of TGD and crypt survivals, namely, α/E and β/E [14]. The reciprocal total dose for crypt survival after any LET increased with an increase in the dose per fraction (Fig. 4A, left panel). The reciprocal total dose for sTGD of X-rays and 20 keV/μm C-ions also slightly increased with an increase in the dose per fraction, while, in contrast, that for the tumor of 77 keV/μm C-ions rather decreased with an increase in dose per fraction (Fig. 4A, right panel). The α terms of the crypt and the tumor apparently increased with an increase in the LET (Fig. 4B, left panel). The increase in the α terms was significantly ($p = 0.0019$) larger for the