The Japan Radiation Research Society

J. Radiat. Res., 45, 303–308 (2004) *Regular Paper*

Differences in pO_2 Peaks of a Murine Fibrosarcoma between Carbon-ion and X-ray Irradiation

Takeshi FUKAWA[1,3]*, Koji TAKEMATSU[2], Kotaro OKA[1,2], Sachiko KOIKE[3], Koichi ANDO[3], Hirosuke KOBAYASHI[4] and Kazuo TANISHITA[1,2]

pO_2 profile/Reoxygenation/HIMAC/Fibrosarcoma.

We measured and compared the oxygen partial pressure (pO_2) profiles in experimental tumors after irradiation with carbon ions and with X-rays. The NFSa fibrosarcomas grown in the hind legs of C3H male mice received isoeffect single doses of carbon ions or X-rays. Coaxial oxygen microelectrodes of high spatial resolution were inserted into the tumor with 20 μm steps by a computerized micromanipulator. The number of pO_2 peaks that reached 15 mmHg were at least 0.45 per 3,000 μm in unirradiated tumors and significantly increased to 1.55 per 3,000 μm as early as day 1 of carbon-ion irradiation ($p < 0.001$). The tumors that received X-ray irradiation also significantly increased pO_2 peaks, but as late as day 3. The time course of pO_2 peak appearance in the present study coincides with a previous report where reoxygenation was measured by paired growth delay assay. The pO_2 peaks appeared selectively in peripheral regions of X-ray irradiated tumors, but they appeared rather homogeneously in the tumor after carbon-ion irradiation. It is concluded that carbon-ion irradiation reoxygenated the NFSa fibrosarcomas earlier in time and deeper in space than the X-ray irradiation did.

INTRODUCTION

Oxygen is one of the most important factors in determining tumor response to radiotherapy. The hypoxic cells are radioresistant to photon.[1,2] The hypoxic tumor cells result from a combination of an inadequate blood supply, such as decreased vascular density and aberrant blood flow, and a greater oxygen demand because of relatively high proliferation rates.[3]

One strategy against the hypoxic tumor cells is to use heavy particles that depend less on the tissue pO_2 than on photons to kill tumor cells.[4] The clinical trial of heavy ion therapy using carbon ions started at the National Institute of Radiological Sciences (NIRS) Japan in 1994[5] and at Gesellschaft für Schwerionenforschung (GSI) Germany in 1997.[6]

The reoxygenation that hypoxic cells become oxygenated after irradiation[7] is also important for carbon-ion therapy because the OER (oxygen enhancement ratio) of carbon ions clinically used is larger than 1.0.[8] A few papers refer to any difference of reoxygenation between high and low LET (linear energy transfer) irradiation and report that reoxgenation after carbon ions is faster than photon irradiation in some tumors.[9,10] Ando *et al.*[10] compared the time course of the reoxygenation, which is measured by tumor growth delay assay in NFSa fibrosarcomas after γ-ray, with those after carbon-ion irradiation and report that although the tumor growth delay is the same between both groups, reoxygenation in the NFSa fibrosarcomas is observed earlier after irradiation with carbon ions compared with γ-rays. They have investigated neither the change of oxygen tension in tumors, nor the mechanism of the accelerated reoxygenation after carbon-ion irradiation.

The correlations between the reoxygenation and the change of oxygen tension have been investigated in many experimental tumors after the low LET irradiation.[11–14] Numerous researches using the oxygen electrodes have reported an increase of the mean and/or median pO_2 values or a decrease of the percentage frequency of pO_2 below either 2.5 or 5 mmHg in tumors after irradiation.[11,12,15] However, the values such as mean (and/or median) pO_2 and the percentage frequency of pO_2 below 2.5 mmHg present no microregional information of a tumor. Taking into account the microregional heterogeneity of oxygen delivery,[16–18] we directly measured the pO_2 profiles in tumors by using a high spatial

*Corresponding author: Phone: +81-43-206-3235,
Fax: +81-43-206-4149,
E-mail: fukawa@nirs.go.jp

[1]Institute of Biomedical Engineering, and [2]Department of System Design Engineering, Keio University, 3-14-1 Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan; [3]Division of Heavy-ion Radiobiology Research Group, National Institute of Radiological Science, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan; and [4]Department of Medicine, Kitasato University School of Medicine, Kitasato 1-15-1, Sagamihara 228-8555, Japan.

NII-Electronic Library Service

The Japan Radiation Research Society

resolution microcoaxial oxygen electrode and compared the change of the oxygen tension in tumors between carbon-ion and X-ray irradiation.

MATERIALS AND METHODS

Mice and tumor

C3H/HeMsNrsf male mice aged 12–18 weeks were used for this study. They were produced and maintained in the specific pathogen-free facilities at NIRS. The tumor was a syngeneic NFSa fibrosarcoma, and its 16th generation was used. Single-cell suspensions were prepared by enzymatically digesting tumors and then transplanted intramuscularly into the right hind leg of mice 7 through 8 days before irradiation.[19,20] Thirty mice for each group (untreated, X-ray and carbon-ion irradiation) were used for pO_2 measurement experiments. Tumor diameters in the three orthogonal directions (a, b, and c) were measured daily with calipers. Tumor volume was calculated with this the formula: tumor volume = $(abc\pi)/6$. Relative tumor volume was calculated as a ratio of the concerned tumor volume to the tumor volume at day 0.

Irradiation

The carbon ions were accelerated by the HIMAC synchrotron at NIRS up to 290 MeV/u. Irradiation was conducted by the use of horizontal carbon beams of 74 keV/μm in LET with a 6 cm width spread-out Bragg peak (SOBP).[21] The irradiation field was produced by the simultaneous use of an iron collimator and a brass collimator. The dose rate was approximately 3 Gy/min.[10] X-rays were used as a reference beam. They were produced by a 200 kVp X-ray unit (PANTAK 320S, Shimadzu, Kyoto, Japan) of 20 mA with a filtration of 0.5 mm Cu and 0.5 mm Al at a dose rate of 2.4 Gy/min. With pentobarbital anesthesia (50 mg kg^{-1}) and taping, the mice were immobilized on a Lucite plate to place their right hind legs in the irradiation field, and then they received single-dose irradiation. The tumor diameter was 7.5 ± 0.5 mm (mean and range). A tumor growth delay of 8 days was selected here as the isoeffect because this isoeffect was previously used to study reoxygenation in the NFSa fibrosarcoma.[10] The isoeffect doses were 16 Gy and 25.5 Gy for 74 keV/μm carbon ions and X rays, respectively.

pO_2 measurements

We measured the tumor pO_2 by using the coaxial oxygen microelectrode fabricated in our laboratory following the procedure described by Baumgärtl and Lübbers.[22] The recessed Pt cathode and the Ag/AgCl anode separated by the glass capillary were placed closely together, and the tip diameter of the electrode was within less than 10 μm. The microelectrode in 0.9% saline at 37°C and at a polarization voltage of –0.65 V provided a linear relationship between oxygen concentration and an oxygen current at the oxygen concentration ranging from 0.1 to 100%.[22] The oxygen sensitivities of the microelectrode were 3–6 pA/mmHg O_2, and the response time of the microelectrode to achieve 90% of the final reading was 0.47 ± 0.02 s (mean ± SD).

After pentobarbital anesthesia (50 mg kg^{-1}) and taping, the skin over the tumor was punctured with a 26-gauge needle, and the tumor surface was immediately covered with 0.9% saline solution. Rectal temperature was kept at 37°C by a thermostatically controlled heating pad during pO_2 measurements. The number of electrode tracks evaluated in each tumor was 2 to 3, and a total of pO_2 profiles of 20–25 were obtained in 10 tumors per day. The inserted microelectrode was controlled by a computerized micromanipulator (ME-71, Narishige, Tokyo, Japan) and moved toward the tumor center with a step width of 20 μm. The microelectrode was moved once 40 μm forward, then pulled 20 μm backward, which minimized the compression artifact of the current caused by the penetration of the microelectrode. The local reduction current was measured by an electrometer (R8340A, Advantest, Tokyo, Japan) during 1.0 s after the back motion.

The electrodes were calibrated by using a buffered 0.9% saline solution equilibrated with air and with 100% nitrogen at 37°C before and after the pO_2 measurements in tumor tissues. All electrode currents were converted to pO_2 in the tumor by using the formula:

$$pO_2 = pO_2(\text{saline})/(K_{\text{tumor}}/K_{\text{saline}}).$$

All electrode currents were once converted to pO_2(saline) by use of the calibration curve made under the saline buffer. The pO_2(saline) was then divided by the ratio of Krogh's diffusion coefficient for tumor tissue (K_{tumor}) to that for saline buffer (K_{saline}). This division is necessary because the steepness of the calibration curve is proportional to the oxygen conductivity (Krogh's diffusion coefficient) of the medium.[22] The K_{tumor} used here was 1.9×10^{-5} $mlO_2/cm/min/atm$[23], and Krogh's diffusion coefficient for water (4.7×10^{-5} $mlO_2/cm/min/atm$)[24] was substituted for K_{saline}.

Statistical analysis

Statistical comparisons of data were performed by nonparametric analysis by use of the Mann-Whitney *U*-test. A significant criterion of $p < 0.05$ was used.

RESULTS

We measured the tumor diameters after a single irradiation with 16 Gy of 74 keV/μm carbon ions and 25.5 Gy of X-rays (Fig. 1). The unirradiated tumor grew at a relatively uniform rate, and the radiation with either X rays or carbon ions identically delayed tumor growth by 8 days. We selected tumors between day 1 and day 8 of irradiation for pO_2 measurements.

The pO_2 at the tumor surface was almost equal to the partial pressure of oxygen in the air, i.e., 150 mmHg (Fig. 2). The pO_2 decreased rapidly when the microelectrode moved from the tumor surface toward the center and reached the hypoxic/anoxic region ($pO_2 < 5$ mmHg) at a depth ranging from 600 to

NII-Electronic Library Service

The Japan Radiation Research Society

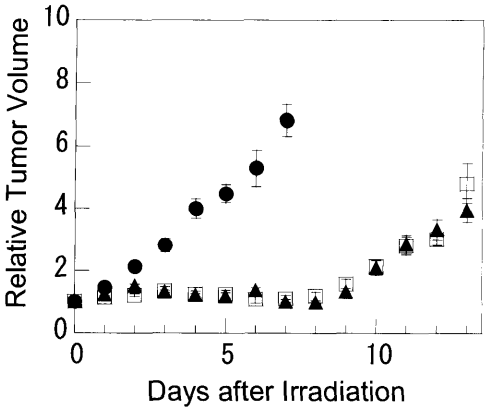

Fig. 1. Volume changes of the NFSa fibrosarcomas after irradiation. Closed circles, closed triangles, and open squares are untreated, X-ray, and carbon-ion irradiated tumors, respectively. The symbols and bars are the mean and SEM calculated from five mice each.

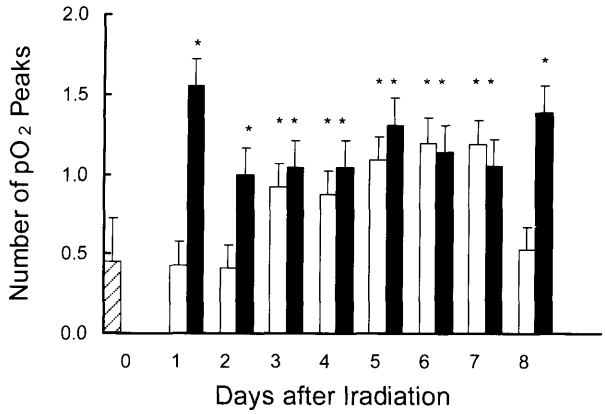

Fig. 3. Time course of the number of pO_2 peaks after irradiation The average number of pO_2 peaks was calculated from 20–25 pO profiles per day for each group. The striped, white, and black bars are untreated, X-ray, or carbon-ion irradiated tumors, respectively. The error bars indicate SEM. The statistical significance ($*p < 0.05$) was obtained between untreated and irradiated tumors.

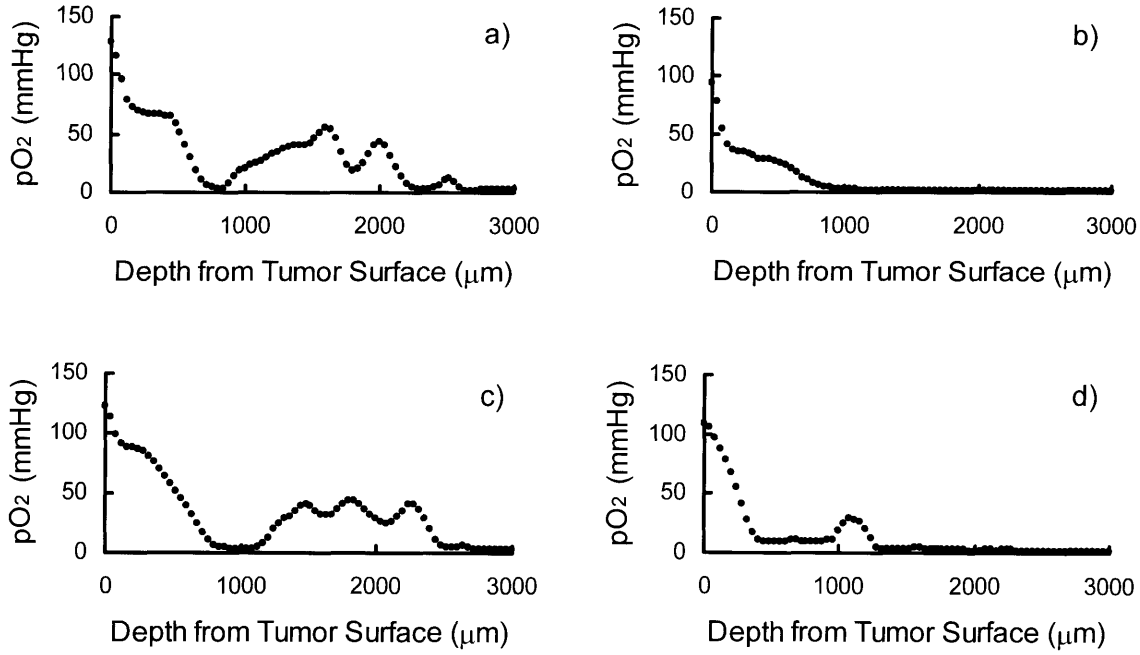

Fig. 2. pO_2 profiles in the NFSa fibrosarcomas after irradiation. (a) and (c) are profiles obtained for a single tumor 1 or 5 days after carbon-ion irradiation, respectively, and (b) and (d) are profiles for another single tumor 1 or 4 days after X-ray irradiation, respectively. The pO_2 profile was collected toward the center from the tumor surface.

1,000 μm. We counted the number of pO_2 peaks that satisfied the following two conditions: (1) 15 mmHg or higher; (2) appearing within the range of 600–3,000 μm depth from the tumor surface. The typical pO_2 profiles after irradiation are shown in Fig. 2. At day 1 of radiation, the number of pO_2 peaks after carbon ions was 3 at the depths of 1,600, 2,000, and 2,520 μm (Fig. 2a), but there were no pO_2 peaks after X-rays (Fig. 2b). One pO_2 peak at 1,100 μm was detected in a tumor that received X-rays 4 days before (Fig. 2d), and the carbon-ion irradiated tumor had 3 pO_2 peaks at 1,500, 1,800, and 2,300 μm (Fig. 2c).

The number of pO_2 peaks was 0.45 in untreated tumors (Fig. 3). One day after irradiation with carbon ions, the number of pO_2 peaks significantly increased to 1.55 ($p < 0.001$, vs. untreated tumors). The increase in the number of pO_2 peaks after carbon-ion irradiation was observed till day 8. For X-ray irradiation, the number of pO_2 peaks increased to 0.92 ($p < 0.05$, vs. untreated tumors) at day 3, and the increase was

NII-Electronic Library Service

The Japan Radiation Research Society

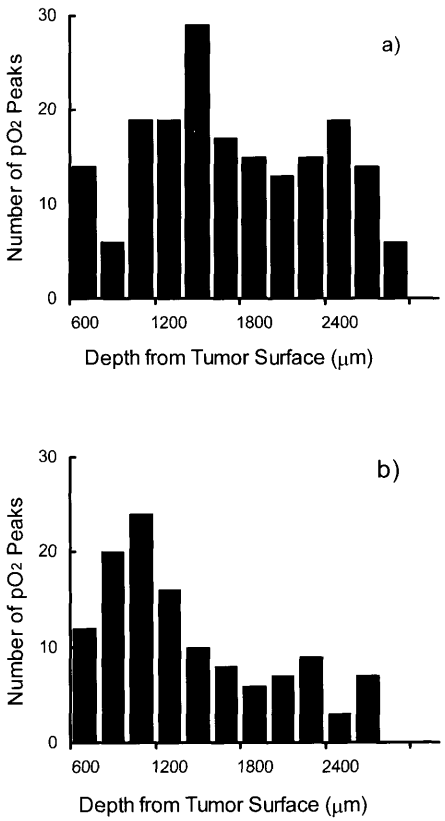

Fig. 4. pO_2 peak distributions in the NFSa fibrosarcomas after irradiation. (a) and (b) are for tumors irradiated with carbon ions or X rays, respectively. A total of 179 (carbon ions) or 175 (X-rays) pO_2 profiles obtained from day 1 to day 8 after irradiation were used for the distributions.

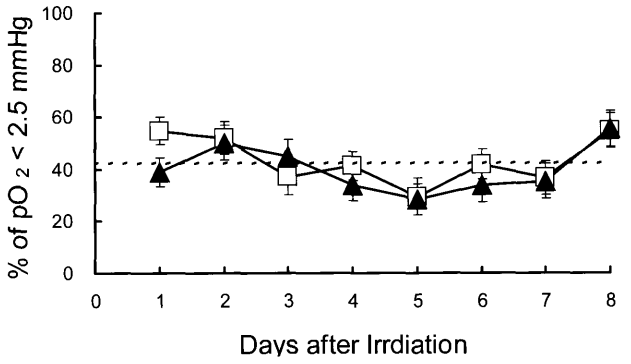

Fig. 5. Time course of the percentage frequency of pO_2 values lower than 2.5 mmHg after irradiation. The closed triangles and open squares are X-ray or carbon-ion irradiated tumors, respectively. The broken line represents the percentage frequency of pO_2 values lower than 2.5 mmHg in untreated tumors. The percentage frequency of the symbols and bars are the mean and SEM calculated from 20–25 pO_2 profiles per day for each group.

detectable till day 7. The number of pO_2 peaks decreased to 0.15 at day 10, a value indistinguishable from untreated tumors (data not shown).

The spatial position of pO_2 peaks in tumors irradiated with carbon ions was deeper than that irradiated with X rays ($p < 0.0001$) (Fig. 4). The depth of the pO_2 peak after carbon ions and X-rays was 1,805 ± 45 and 1,445 ± 55 μm (mean ± SEM), respectively, from the tumor surface. The median depth of the pO_2 peak was 1,720 μm after carbon-ion irradiation, but it was 1,280 μm after X-ray irradiation.

Figure 5 shows the percentage frequency of pO_2 values lower than 2.5 mmHg after irradiation. This frequency was calculated from 120 pO_2 values per pO_2 profile within a range of 600–3,000 μm depth from the tumor surface, and a total of 20–25 profiles per day were obtained in 10 tumors. The percentage frequency of pO_2 values lower than 2.5 mmHg in untreated tumors was 42.3 ± 6.8% (mean ± SEM). There was no statistically significant change in the percentage frequency of pO_2 readings lower than 2.5 mmHg ($p > 0.05$), nor was there any statistically significant change lower than 5 mmHg (data not shown). The mean and median pO_2 also did not change statistically (data not shown).

DISCUSSION

The unirradiated NFSa tumors were under low-oxygen condition so that the percentage of pO_2 below 2.5 mmHg was about 40% (Fig. 5). Using electron paramagnetic spectroscopy, Halpern *et al.* have measured and reported that mean pO_2 is about 3 mmHg for 0.25 g NFSa tumor.[25] Ando *et al.*[26] report the radiological hypoxic cell fraction of the NFSa tumors as 0.1 for the same tumor size as used in the present study. The relationship between the percentage frequency of pO_2 below either 2.5 or 5 mmHg and the hypoxic fraction assessed by radiological assays is not established.[27,28] It is difficult to distinguish between measurements made in clonogenic vs. nonclonogenic cells when polarographic oxygen electrodes are used.

Because our results are similar to the calculated pO_2 profiles using Krogh's cylinder model,[29] the pO_2 peaks detected in the present study are probably produced when the oxygen electrode passes over the vicinity of the functional blood vessel. In the present study, the microcoaxial electrode we used had a high spatial resolution that was materialized by (1) closely placing the working and reference electrode coaxially, and (2) employing a small-tip electrode. Previous studies using the oxygen electrodes have detected no pO_2 peaks in tumors. This is because their spatial resolution is too low to detect the heterogeneities in tumor vascular configuration[30] and HbO_2 saturations.[18] The microregional variability of oxygen delivery is also reported in an experimental tumor model using a dorsal window chamber.[16,17] The presence of pO_2 peaks means that oxygen tension in the NFSa tumor is heterogeneous and microregionally variable.

The pO_2 distribution of tumors after irradiation was different between carbon ions and X rays. Carbon-ion irradiation increased pO_2 peaks faster in time and deeper in space than X-

NII-Electronic Library Service

The Japan Radiation Research Society

ray irradiation, even though tumor growth after irradiation was the same between the two radiation qualities. The oxygen tension of tumors changes after single or fractionated doses of photon radiation to tumors in experimental animals[11,12,15] and in patients,[31,32] which is usually assessed by polarography oxygen electrodes. Most of the experimental studies using microelectrodes measure pO_2 at one or two times after irradiation, and use the percentage frequency of pO_2 below either 2.5 or 5 mmHg in tumors as a cutoff determining the hypoxic fraction (HF_{pO2}). Vaupel *et al.*[11] and Koutcher *et al.*[12] reported a marked reduction of the HF_{pO2} in mouse mammary carcinomas a few days after irradiation with single doses from 30 to 60 Gy, which indicates reoxygenation. In contrast, Zewietz *et al.*[15] report that the HF_{pO2} rather increases with radiation doses of more than 45 Gy, which indicates a promotion of hypoxia. In the present study, the HF_{pO2} did not change significantly after irradiation regardless of radiation qualities (Fig. 5).

The time of increase in the pO_2 peaks was earlier for carbon ions than for X-rays ($p < 0.05$) (Fig. 3). Ando *et al.*[10] have showed that the NFSa tumors reoxygenate 4 days after 30 Gy of gamma rays, but they reoxygenate at 1 day after a 74 keV/μm carbon-ion dose of 16 Gy. The time course of pO_2 peak appearance in the present study coincides with that of reoxygenation reported by Ando *et al.* Oya *et al.*[9] report that the reoxygenation occurs more rapidly after carbon-ion than X-ray irradiation for SCCVII and SCCVII-variant-1 tumors, whereas a quite similar reoxygenation is observed for the EMT6 tumors after X-ray and carbon-ion irradiation.

Several possible mechanisms of reoxygenation have been suggested. They include reduced oxygen consumption by radiation-damaged cells,[33] cell loss leading to tumor shrinkage,[7] migration of hypoxic cells to oxygenated areas[7] and improved microcirculation.[1] The increase in pO2 peaks after irradiation (Fig. 3) support the assumption that the reoxygenation is caused by increased functional blood vessels.[34] The pO_2 peaks appeared selectively in peripheral regions after X-ray irradiation, but rather homogeneously in the tumor after carbon-ion irradiation (Fig. 4). The pO_2 is reported higher in the periphery than in the center of the mouse mammary adenocarcinomas MTG-B.[13] The interstitial fluid pressure (IFP) is also higher in the center than in the periphery, and it increases with the size of the tumor.[35] Tozer *et al.*[35] report that the blood flow in untreated tumors is heterogeneous and decreases toward the center. Numerous papers report the changes of oxygen delivery in normal tissues[36] and tumor tissues[37] after radiation. After photon irradiation, IFP significantly decreases at the tumor center,[38] and blood flow tends to increase toward the center.[35] Because cell killing by carbon ions depends less on oxygen than by photons, the distribution of radiation-induced cell death would be more homogeneous for carbon ions than for photons. The carbon ions may reduce the IFP regardless of tumor periphery or center, but X-rays may reduce the IFP preferentially in tumor periphery. The IFP reduction would open any nonfunctional or compressed vessels, resulting in an increased tumor blood flow and pO_2 peaks.

ACKNOWLEDGEMENTS

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, NIRS. This work was supported in part by a Grant-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by the Special Coordination Funds for Research Project with Heavy Ions at the National Institute of Radiological Sciences-Heavy-ion Medical Accelerator in Chiba (NIRS-HIMAC).

REFERENCES

1. Brown, J. M. (1979) Evidence for acutely hypoxic cells in mouse tumours, and a possible mechanism of reoxygenation. Br. J. Radiol. **52**: 650–656.
2. Kallinowski, F., Zander, R., Hoeckel, M. and Vaupel, P. (1990) Tumor tissue oxygenation as evaluated by computerized-pO2- histography. Int. J. Radiat. Oncol. Biol. Phys. **19**: 953–961.
3. Vaupel, P., Kallinowski, F. and Okunieff, P. (1989) Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. Cancer Res. **49**: 6449–6465.
4. Raju, M. R., Amols, H. I., Bain, E., Carpenter, S. G., Cox, R.A. and Robertson, J. B. (1978) A heavy particle comparative study. Part III: OER and RBE. Br. J. Radiol. **51**: 712–719.
5. Tsujii, H., Morita, S., Miyamoto, T., Mizoe, J., Mukai, M., Nakano, T., Kato, H., Kamada, T., Ishikawa, A. and Matsuoka, Y. (1997) Preliminary results of phase I/II carbon-ion therapy at the National Institute of Radiological Sciences. J. Brachyther. Int. **13**: 1–8.
6. Schulz-Ertner, D., Haberer, T., Scholz, M., Thilmann, C., Wenz, F., Jakel, O., Kraft, G., Wannenmacher, M. and Debus, J. (2002) Acute radiation-induced toxicity of heavy ion radiotherapy delivered with intensity modulated pencil beam scanning in patients with base of skull tumors. Radiother. Oncol. **64**: 189–195.
7. Kallman, R. F. (1972) The phenomenon of reoxygenation and its implications for fractionated radiotherapy. Radiology **105**: 135–142.
8. Furusawa, Y., Fukutsu, K., Aoki, M., Itsukaichi, H., Eguchi-Kasai, K., Ohara, H., Yatagai, F., Kanai, T. and Ando, K. (2000) Inactivation of aerobic and hypoxic cells from three different cell lines by accelerated (3)He-, (12)C- and (20)Ne-ion beams. Radiat. Res. **154**: 485–496.
9. Oya, N., Sasai, K., Shibata, T., Takagi, T., Shibuya, K., Koike, S., Nojima, K., Furusawa, Y., Ando, K. and Hiraoka, M. (2001) Time course of reoxygenation in experimental murine tumors after carbon-beam and X-ray irradiation. J. Radiat. Res. **42**: 131–141.
10. Ando, K., Koike, S., Ohira, C., Chen, Y. J., Nojima, K., Ando,

NII-Electronic Library Service

The Japan Radiation Research Society

S., Ohbuchi, T., Kobayashi, N., Shimizu, W. and Urano, M. (1999) Accelerated reoxygenation of a murine fibrosarcoma after carbon-ion radiation. Int. J. Radiat. Biol. **75**: 505–512.
11. Vaupel, P., Frinak, S. and O'Hara, M. (1984) Direct measurement of reoxygenation in malignant mammary tumors after a single large dose of irradiation. Adv. Exp. Med. Biol. **180**: 773–782.
12. Koutcher, J. A., Alfieri, A. A., Devitt, M. L., Rhee, J. G., Kornblith, A. B., Mahmood, U., Merchant, T. E. and Cowburn, D. (1992) Quantitative changes in tumor metabolism, partial pressure of oxygen, and radiobiological oxygenation status postradiation. Cancer Res. **52**: 4620–4627.
13. O'Hara, J. A., Goda, F., Liu, K. J., Bacic, G., Hoopes, P. J. and Swartz, H. M. (1995) The pO2 in a murine tumor after irradiation: an *in vivo* electron paramagnetic resonance oximetry study. Radiat. Res. **144**: 222–229.
14. Goda, F., O'Hara, J. A., Rhodes, E. S., Liu, K. J., Dunn, J. F., Bacic, G. and Swartz, H. M. (1995) Changes of oxygen tension in experimental tumors after a single dose of X-ray irradiation. Cancer Res. **55**: 2249–2252.
15. Zywietz, F., Reeker, W. and Kochs, E. (1995) Tumor oxygenation in a transplanted rat rhabdomyosarcoma during fractionated irradiation. Int. J. Radiat. Oncol. Biol. Phys. **32**: 1391–1400.
16. Dewhirst, M. W., Ong, E. T., Klitzman, B., Secomb, T. W., Vinuya, R. Z., Dodge, R., Brizel, D. and Gross, J. F. (1992) Perivascular oxygen tensions in a transplantable mammary tumor growing in a dorsal flap window chamber. Radiat. Res. **130**: 171–182.
17. Helmlinger, G., Yuan, F., Dellian, M. and Jain, R. K. (1997) Interstitial pH and pO2 gradients in solid tumors *in vivo*: high-resolution measurements reveal a lack of correlation. Nat. Med. **3**: 177–182.
18. Fenton, B. M., Raubertas, R. F. and Boyce, D. J. (1995) Quantification of micro-regional heterogeneities in tumor oxygenation using intravascular HbO2 saturations. Radiat. Res. **141**: 49–56.
19. Ando, K., Koike, S. and Sato, S. (1992) Nonlinear survival curves for cells of solid tumors after large doses of fast neutrons and gamma rays. Radiat. Res. **131**: 157–161.
20. Koike, S., Ando, K., Oohira, C., Fukawa, T., Lee, R., Takai, N., Monobe, M., Furusawa, Y., Aoki, M., Yamada, S., Shimizu, W., Nojima, K. and Majima, H. (2002) Relative biological effectiveness of 290 MeV/u carbon ions for the growth delay of a radioresistant murine fibrosarcoma. J. Radiat. Res. **43**: 247–255.
21. Kanai, T., Kohno, T., Minohara, S., Sudou, M., Takada, E., Soga, F., Kawachi, K. and Fukumura, A. (1993) Dosimetry and measured differential W values of air for heavy ions. Radiat. Res. **135**: 293–301.
22. Baumgartl, H. and Lubbers, D. W. (1983) Microcoaxial needle sensor for polarographic measurement of local O_2 pressure in the cellular range of living tissue. Its construction and properties. In: Polarographic Oxygen Sensors, Eds. E. Gnaiger, and H. Forstner, pp. 37–65, Springer-Verlag, Berlin, Heidelberg, New York.
23. Grote, J., Susskind, R. and Vaupel, P. (1977) Oxygen diffusivity in tumor tissue (DS-carcinosarcoma) under temperature conditions within the range of 20–40 degrees C. Pflügers. Arch. **372**: 37–42.
24. Grote, J. (1967) Die Sauerstoffdiffusionskonstanten im Lungengewebe und Wasser und ihre Temperaturabhangigkeit. Pflügers. Arch. Gesamte. Physiol. Menschen. Tiere **295**: 245–254.
25. Halpern, H. J., Yu, C., Peric, M., Barth, E., Grdina, D. J. and Teicher, B. A. (1994) Oxymetry deep in tissues with low-frequency electron paramagnetic resonance. Proc. Natl. Acad. Sci. U.S.A. **91**: 13047–13051.
26. Ando, K., Koike, S., Shikita, M., Hayata, I., Otsu, H. and Satoh, S. (1988) Radiosensitivity of late recurrences following radiotherapy of murine fibrosarcomas. Radiat. Res. **113**: 334–345.
27. Horsman, M. R., Khalil, A. A., Siemann, D. W., Grau, C., Hill, S. A., Lynch, E. M., Chaplin, D. J. and Overgaard, J. (1994) Relationship between radiobiological hypoxia in tumors and electrode measurements of tumor oxygenation. Int. J. Radiat. Oncol. Biol. Phys. **29**: 439–442.
28. Kavanagh, M. C., Sun, A., Hu, Q. and Hill, R. P. (1996) Comparing techniques of measuring tumor hypoxia in different murine tumors: Eppendorf pO2 Histograph, [3H]misonidazole binding and paired survival assay. Radiat. Res. **145**: 491–500.
29. Vaupel, P. and Thews, G. (1974) PO2 distribution in tumor tissue of DS-carcinosarcoma. Oncology **30**: 475–484.
30. Fenton, B. M., Lord, E. M. and Paoni, S. F. (2001) Intravascular HBO(2) saturations, perfusion and hypoxia in spontaneous and transplanted tumor models. Int. J. Cancer **93**: 693–698.
31. Cooper, R. A., West, C. M., Logue, J. P., Davidson, S. E., Miller, A., Roberts, S., Statford, I. J., Honess, D. J. and Hunter, R. D. (1999) Changes in oxygenation during radiotherapy in carcinoma of the cervix. Int. J. Radiat. Oncol. Biol. Phys. **45**: 119–126.
32. Lyng, H., Tanum, G., Evensen, J. F. and Rofstad, E. K. (1999) Changes in oxygen tension during radiotherapy of head and neck tumours. Acta Oncol. **38**: 1037–1042.
33. Olive, P. L. (1994) Radiation-induced reoxygenation in the SCCVII murine tumour: evidence for a decrease in oxygen consumption and an increase in tumour perfusion. Radiother. Oncol. **32**: 37–46.
34. Rofstad, E. K. (1989) Hypoxia and reoxygenation in human melanoma xenografts. Int. J. Radiat. Oncol. Biol. Phys. **17**: 81–89.
35. Tozer, G. M., Myers, R. and Cunningham, V. J. (1991) Radiation-induced modification of blood flow distribution in a rat fibrosarcoma. Int. J. Radiat. Biol. **60**: 327–334.
36. Kiani, M. F., Ansari, R. and Gaber, M. W. (2003) Oxygen delivery in irradiated normal tissue. J. Radiat. Res. **44**: 15–21.
37. Bussink, J., Kaanders, J. H., Rijken, P. F., Raleigh, J. A. and Van der Kogel, A. J. (2000) Changes in blood perfusion and hypoxia after irradiation of a human squamous cell carcinoma xenograft tumor line. Radiat. Res. **153**: 398–404.
38. Znati, C. A., Rosenstein, M., Boucher, Y., Epperly, M. W., Bloomer, W. D. and Jain, R. K. (1996) Effect of radiation on interstitial fluid pressure and oxygenation in a human tumor xenograft. Cancer Res. **56**: 964–968.

Received on November 14, 2003
1st Revision on February 11, 2004
2nd Revision on February 23, 2004
Accepted on February 23, 2004

NII-Electronic Library Service