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Effectiveness of Monoenergetic and Spread-Out Bragg Peak Carbon-Ions for Inactivation of Various Normal and Tumour Human Cell Lines

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Human cell line/Carbon-ion beams/Cell inactivation/Relative Biological Effectiveness (RBE)/Linear Energy Transfer (LET).

This work aimed at measuring cell-killing effectiveness of monoenergetic and Spread-Out Bragg Peak (SOBP) carbon-ion beams in normal and tumour cells with different radiation sensitivity. Clonogenic survival was assayed in normal and tumour human cell lines exhibiting different radiosensitivity to X- or γ-rays following exposure to monoenergetic carbon-ion beams (incident LET 13–303 keV/μm) and at various positions along the ionization curve of a therapeutic carbon-ion beam, corresponding to three dose-averaged LET (LET_d) values (40, 50 and 75 keV/μm). Chinese hamster V79 cells were also used. Carbon-ion effectiveness for cell inactivation generally increased with LET for monoenergetic beams, with the largest gain in cell-killing obtained in the cells most radioresistant to X- or γ-rays. Such an increased effectiveness in cells less responsive to low LET radiation was found also for SOBP irradiation, but the latter was less effective compared with monoenergetic ion beams of the same LET. Our data show the superior effectiveness for cell-killing exhibited by carbon-ion beams compared to lower LET radiation, particularly in tumour cells radioresistant to X- or γ-rays, hence the advantage of using such beams in radiotherapy. The observed lower effectiveness of SOBP irradiation compared to monoenergetic carbon beam irradiation argues against the radiobiological equivalence between dose-averaged LET in a point in the SOBP and the corresponding monoenergetic beams.

INTRODUCTION

Carbon-ions play a special role in hadrontherapy and are the second most frequent ions, after protons, used for this purpose. Three centres currently treat patients with carbon-ions: the HIMAC in Chiba, Japan;[1,2)] the HIBMC in Hyogo, Japan;[3)] the GSI in Darmstadt, Germany.[4,5)]

The potential clinical advantages of hadron beams pertain to the energy distribution in the traversed matter, which is localized along the particle track and differs greatly from that generated by photon beams (X- and γ-rays) at both macroscopic and microscopic scale. The increase in ionization near the path-end (Bragg peak) ensures that most of the dose is delivered in depth.

The biological effects of charged hadrons have been mostly studied in biological systems *in vitro*.[6,7)] A great deal of work on carbon-ions has been done at the hadrontherapy centres, while some low-energy studies have been performed at other nuclear physics laboratories.

From radiobiological studies, it is expected that carbon-ions, like other densely ionizing radiation, show a high RBE for cell-killing in cells that are repair proficient and radiore-

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sistant to X- or γ-rays.[8–11)]

Clinical evidence is accumulating that confirms an advantage of carbon-ion beam therapy for sparsely ionizing radiation resistant tumours,[12)] as in several types of brain tumour.[13)] However, carbon-ions (as well as other densely ionizing radiations) imply a greater risk in normal tissue for late effects than X- and γ-rays or protons.[14,16–21)] The variation of beam quality with penetration depth due to the carbon fragmentation may result in RBE changes for cell-killing that can be exploited for clinical benefit. Preclinical studies are therefore fundamental for assessing these radiobiological properties at different depths of the irradiated tissue.

Thus far, inactivation has been studied in cell types exposed to carbon beams that were either monoenergetic or had a defined average LET[15,16, 22,23)] or along the therapeutic SOBP.[22,24)] It should be considered that each point of the SOBP-irradiated tissue is exposed to a mixed radiation field. This is also the case when a beam with a defined average LET is obtained by degradation through absorbers of an initially higher energy beam, due to the interaction of carbon-ions with the absorber. Understanding the relationship between the biologically relevant effects induced by monoenergetic carbon-ions and those induced by SOBP or degraded beams, and how this relationship depends on cell radiosensitivity is essential for a radiobiological comparison of different beams as well as for improving radiobiological models to be used in treatment planning.[25,26)]

In this work, we studied carbon-ion induced inactivation in four human cell lines of normal and tumour origin, with different radiosensitivities to γ-rays. Radioresistant Chinese hamster V79 cells were also used because they are known to be very responsive to radiation quality changes.[27–29)] Cells were irradiated either with monoenergetic carbon beams or at different positions along a carbon ion beam SOBP.

MATERIALS AND METHODS

Cell lines and cultures

Two out of the four human cell lines used in addition to the aforementioned V79 cells were derived from tumour tissues (SCC25 and SQ20B) and two from normal tissues (HF19 and H184B5 F5-1M/10 hereafter called M/10). These cell lines have been extensively employed in experiments with X- and γ-rays, showing different radiation sensitivities. [11)]

SCC25 and SQ20B cell lines were derived from human epithelial tumours of the tongue and of the larynx, respectively,[30)] kindly donated by Dr. E. A. Blakely. Cells were grown in D-MEM:F12 (75:25) supplemented with 0.4 μg/ml hydrocortisone and 20% foetall calf serum. Under these conditions, the Plating Efficiency (PE) was ~40% for SCC25 and ~60% for SQ20B, and the doubling time T_d, evaluated from the growth curve, was (24 ± 2) h for both lines.

M/10 cell line is a sub-clone taken from a primary culture of the human mammary epithelial cell line H184B,[31)] by courtesy of Dr. T. C. Yang. These cells, grown in α-MEM with 10% foetall calf serum, had a PE of ~30% and a T_d of (28 ± 2) h.

HF19 is a lung fibroblast cell line derived from a female foetus[32)] donated by Dr. J. Tacker. These cells were cultured in Eagle's MEM medium plus 10% foetall calf serum, yielding a PE of about 16% and a T_d of (24 ± 1) h.

Chinese hamster V79 cells were grown in Eagle's MEM medium (10% foetall calf serum) with a PE of ~90% and a T_d of (13 ± 1) h.

Irradiation with carbon-ions and reference γ-rays

Carbon-ions of the lowest energies, namely 4.5, 6.7 and 19 MeV/amu, were used at the Tandem-ALPI facility of the Laboratori Nazionali di Legnaro-Istituto Nazionale di Fisica Nucleare (LNL-INFN), Legnaro, Italy. The radiobiological beam line set-up is similar to that for proton and other light ion irradiation at the 7 MV Van de Graaff CN accelerator.[33,34)] Briefly, the carbon ion beam passes through two diffusing gold foils (each 2.2 mg/cm^2 thick) and is extracted in air through an aluminized Mylar window (10 μm thick). After 1 cm in air and a Mylar foil used as base of irradiation vessel, the beam reaches the cell layer. Beam dosimetry was accomplished before each experiment by two Silicon Surface-Barrier Detectors (SSBD) located along the beam line, counting the ions scattered by the gold foils. The system was calibrated with a third SSBD placed in air at the same position as the cells.

The Superconductive Cyclotron of the Laboratori Nazionali del Sud-Istituto Nazionale di Fisica Nucleare (LNS-INFN), Catania, Italy, provides 59 MeV/amu carbon-ion beams through a beam line developed for ocular proton therapy.[35)] The beam exits the vacuum line through a 50 μm Kapton window. The extracted beam is then defined by three 25-mm diameter collimators. Dosimetry was performed using an ionization chamber calibrated with a plane-parallel Markus chamber located at the position of the cell sample.

For irradiation at Legnaro and Catania, cells were plated in on-purpose built stainless steel cylinders of 13 mm in diameter having a Mylar base of 6 or 52 μm thickness, respectively.[33)]

At the HIMAC of the National Institute for Radiological Science (NIRS), Japan, irradiations were performed with monoenergetic and SOBP carbon-ion beams in the biology cave where the beam delivery system is similar to the horizontal therapeutic beam line. Monoenergetic beams, whose diameter was about 10 cm, were delivered with energy of 135 or 290 MeV/amu. The Bragg curve was measured prior to each experiment. Dosimetry was carried out with an ionization chamber. SOBP irradiations were performed at three different positions along the ionization curve of a 290 MeV/amu carbon beam whose Bragg Peak was modulated to a 6 cm width. The beam was spread out by a ridge filter and

positions along the SOBP were selected by interposing Polymethyl-methacrylate (PMMA) absorbers. The chosen positions corresponded to water-equivalent depths of 84, 111 and 131 mm from the entrance (positions A, B, and C in Fig. 1). The corresponding dose-averaged LET_d values were 40, 50, and 75 keV/μm. LET_d, defined as:

$$LET_d = \frac{\int L^2 f(L) dL}{\int L f(L) dL}$$

with *f(L)* the LET distribution, was evaluated by a calculation code where fragmentation of nuclei is accounted for.[36] Depth-dose distribution was measured with an ionization chamber. Details on the radiobiological irradiation conditions at this facility can be found in Kanai *et al.*.[25] For both monoenergetic and SOBP beams, cells were irradiated in standard T-25 flasks.

Homogeneity of all beams was checked with CR39 detectors and GAFCHROMIC films; in addition, we also used KODAK X-ray film, X-Omat TL at the HIMAC.

The parameters of the carbon-ion beams are reported in Table 1.

Irradiation with reference γ-rays was carried out using ^{60}Co and/or ^{137}Cs sources, depending on their availability. Tests performed to compare the inactivation effectiveness of these two sources did not show significant differences on the same cell line (see Results). Cells were plated and irradiated in T-25 flasks, and the electronic equilibrium was ensured by the plastic wall of the flasks and by a suitable thickness of medium.

All irradiations (carbon-ions and γ-rays) were carried out in air at room temperature, in the dose range 0.25–14 Gy (depending on the cell radiation sensitivity) and at a dose rate of about 1 Gy/min.

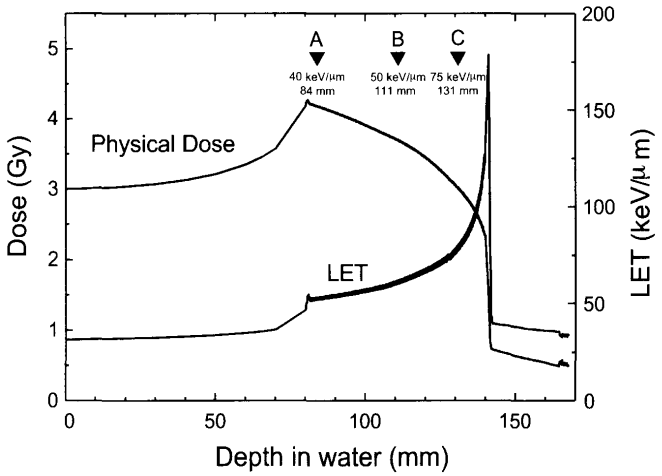

Fig. 1. SOBP and the different positions for cell irradiation.

Cell inactivation

Cell inactivation was measured as reproductive cell death using a colony-forming assay. After irradiation, cells were trypsinised, counted, diluted and plated into flasks or dishes as appropriate. After a period of incubation at 37°C in a 5% CO_2 atmosphere varying from 7 to 18 days, depending on the cell line, cells were fixed and stained. Colonies with more than 50 cells were considered as survivors.

Analysis of the cell inactivation data

Cell surviving fractions, S(D), were evaluated as the ratio between the measured plating efficiency at dose D, PE(D), and the extrapolated plating efficiency to 0 Gy, $PE(0)_{extr.}$. This was evaluated by fitting the function

$$PE(D) = PE(0)_{extr} \exp(-\alpha D - \beta D^2)$$

to the experimental plating efficiencies measured at the various doses (including D = 0 Gy).

Independent experiments were carried out for each cell line and each LET. Cell surviving fraction S(D) at each dose was evaluated as the mean from the independent experiments

Table 1. Monoenergetic carbon-ion beam parameters

Facility	Incident energy* (MeV/n)	Incident LET§ (keV/μm)	Range§ (mm)
HIMAC, NIRS	290	13	170
HIMAC, NIRS	135	24	46
CATANA, INFN-LNS	59	39	11
ALPI, INFN-LNL	19	94	1.2
Tandem, INFN-LNL	6.7	222	0.2
Tandem, INFN-LNL	4.5	303	0.1

* Peak value of the measured ion beam energy incident on the cell layer.
§ Evaluated from the ICRU tables for MS20 tissue (ICRU 1993).

with its standard error. Linear and quadratic parameters α and β were determined by the best fit of the equation:

$$S(D) = \exp(-\alpha D - \beta D^2)$$

When β was found consistent with zero, the fitting procedure was repeated with α as the only free parameter.

RBE calculation

We report three different evaluations of RBE defined as the ratio between the dose of γ-rays and carbon-ions at a given survival level. The first one, RBE_{α}, calculated as α/α_{γ}, is representative of the carbon-ion effectiveness at low doses; the second one, RBE(2Gy, γ), calculated at the cell inactivation level induced by 2 Gy of γ-rays, can be regarded as more relevant for radiation therapy being the RBE corresponding to the typical dose used in fractionated-dose protocols. Finally, the RBE at the 10% survival level is also reported.

RESULTS

Cell inactivation by γ-rays and monoenergetic carbon-ions

Our results with γ-rays confirm the different sensitivities of the human cell lines used in this study. In increasing order of responsiveness, the SQ20B cells were the most resistant, followed by the M/10, the SCC25 and the HF19 cells (Fig. 2 and Table 2). The two γ-rays sources used were shown to

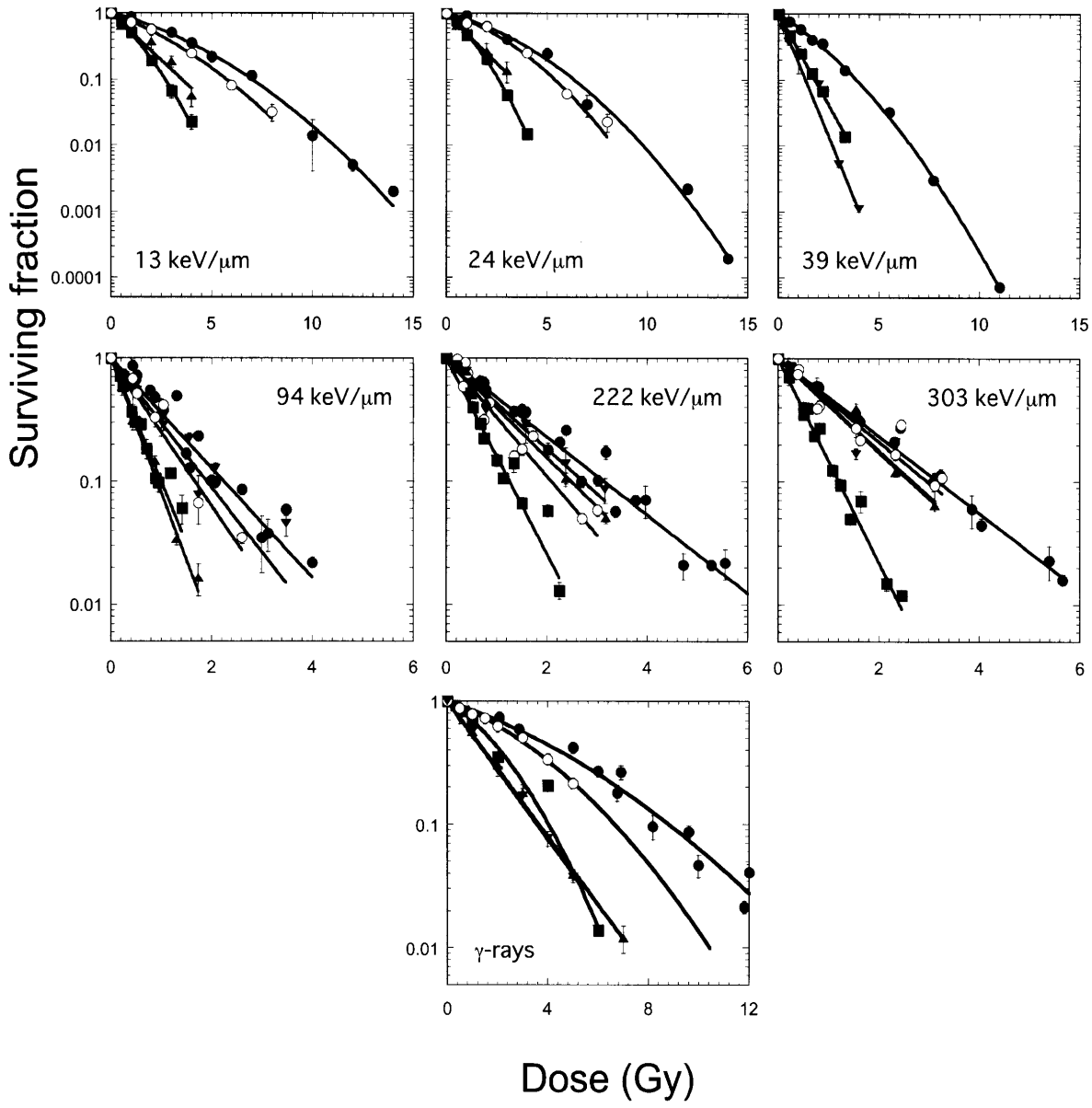

Fig. 2. Cell inactivation by γ-rays and monoenergetic carbon-ions at different LET values. (●)SQ20B; (■)M10; (▲)SCC25; (▼)HF19; (○) V79.

Table 2. Summary of cell survival parameters and RBE values after monoenergetic carbon-ion irradiation

Cell line	LET (keV/μm)	α (Gy^{-1})	β (Gy^{-2})	SF (2 Gy)	RBE (α/α_γ)	RBE (2 Gy,γ)	RBE (10%)
Human							
HF19	(γ-rays)	0.64 ± 0.02		0.275 ± 0.008			
	39	1.71 ± 0.02		0.033 ± 0.001	2.65 ± 0.07	2.65 ± 0.03	2.67 ± 0.09
	94	1.21 ± 0.02		0.089 ± 0.004	1.87 ± 0.06	1.87 ± 0.04	1.89 ± 0.07
	222	0.84 ± 0.06		0.185 ± 0.023	1.31 ± 0.10	1.31 ± 0.10	1.32 ± 0.11
	303	0.86 ± 0.03		0.180 ± 0.010	1.33 ± 0.05	1.33 ± 0.04	1.34 ± 0.06
M/10	(γ-rays)	0.30 ± 0.05	0.068 ± 0.009	0.420 ± 0.044			
	13	0.61 ± 0.09	0.089 ± 0.034	0.205 ± 0.047	2.06 ± 0.46	1.66 ± 0.20	1.51 ± 0.21
	24	0.58 ± 0.10	0.119 ± 0.027	0.195 ± 0.044	1.94 ± 0.46	1.66 ± 0.20	1.55 ± 0.19
	39	1.27 ± 0.02		0.079 ± 0.004	4.26 ± 0.71	2.93 ± 0.05	2.23 ± 0.16
	94	2.30 ± 0.06		0.010 ± 0.001	7.69 ± 1.29	5.29 ± 0.13	4.02 ± 0.31
	222	1.83 ± 0.03		0.026 ± 0.002	6.11 ± 1.02	4.21 ± 0.07	3.19 ± 0.24
	303	1.91 ± 0.03		0.022 ± 0.001	6.38 ± 1.06	4.39 ± 0.06	3.33 ± 0.24
SCC25	(γ-rays)	0.63 ± 0.02		0.281 ± 0.009			
	13	0.66 ± 0.04		0.270 ± 0.023	1.03 ± 0.07	1.03 ± 0.07	1.03 ± 0.07
	24	0.69 ± 0.08		0.250 ± 0.041	1.09 ± 0.13	1.09 ± 0.13	1.09 ± 0.13
	94	2.51 ± 0.06		0.007 ± 0.001	3.95 ± 0.13	3.95 ± 0.09	3.95 ± 0.13
	222	0.93 ± 0.02		0.157 ± 0.007	1.46 ± 0.05	1.46 ± 0.04	1.46 ± 0.05
	303	0.87 ± 0.04		0.174 ± 0.007	1.38 ± 0.05	1.38 ± 0.03	1.38 ± 0.05
SQ20B	(γ-rays)	0.16 ± 0.01	0.012 ± 0.001	0.693 ± 0.014			
	13	0.20 ± 0.02	0.018 ± 0.002	0.628 ± 0.026	1.23 ± 0.15	1.23 ± 0.10	1.23 ± 0.08
	24	0.16 ± 0.03	0.031 ± 0.002	0.635 ± 0.033	1.04 ± 0.17	1.19 ± 0.11	1.37 ± 0.08
	39	0.44 ± 0.02	0.039 ± 0.002	0.354 ± 0.016	2.78 ± 0.22	2.58 ± 0.12	2.24 ± 0.10
	94	1.02 ± 0.01		0.129 ± 0.004	6.45 ± 0.40	5.59 ± 0.08	3.87 ± 0.14
	222	0.73 ± 0.01		0.231 ± 0.004	4.61 ± 0.28	3.99 ± 0.04	2.77 ± 0.10
	303	0.72 ± 0.01		0.236 ± 0.005	4.54 ± 0.29	3.94 ± 0.06	2.73 ± 0.10
Rodent							
V79	(γ-rays)	0.18 ± 0.05	0.025 ± 0.011	0.635 ± 0.060			
	13	0.25 ± 0.02	0.026 ± 0.007	0.543 ± 0.043	1.44 ± 0.44	1.29 ± 0.15	1.16 ± 0.16
	24	0.30 ± 0.03	0.025 ± 0.004	0.499 ± 0.021	1.69 ± 0.47	1.46 ± 0.08	1.25 ± 0.13
	94	1.37 ± 0.03		0.064 ± 0.004	7.80 ± 2.10	6.05 ± 0.13	3.98 ± 0.38
	222	1.10 ± 0.02		0.111 ± 0.004	6.25 ± 1.68	4.85 ± 0.01	3.19 ± 0.30
	303	0.78 ± 0.02		0.212 ± 0.009	4.40 ± 1.19	3.42 ± 0.09	2.25 ± 0.22

yield the same inactivation results for a given cell line: The best fit parameters of the survival curves obtained irradiating the radioresistant SQ20B and the radiosensitive HF19 cells with ^{60}Co or ^{137}Cs γ-rays were the same within experimental errors (SQ20B: $\alpha = 0.15 \pm 0.01$ vs 0.17 ± 0.02 Gy^{-1} and $\beta = 0.012 \pm 0.001$ vs 0.011 ± 0.001 Gy^{-2}; HF19: $\alpha = 0.64 \pm 0.02$ vs 0.65 ± 0.02 Gy^{-1}).

The dose-response curves for cell inactivation after carbon-ion irradiations are shown in Fig. 2. The best fit parameters are reported in Table 2 together with the surviv-

ing fraction at 2 Gy (SF2) and the three RBE evaluations.

With increasing LET the effectiveness of carbon-ions for inducing cell inactivation increases as shown by clonogenic survival curves from the most radioresistant human cell lines (SQ20B, M/10), as well as the rodent V79 cells, which tend to lose their shoulder at low doses. At LET values higher than 94 keV/μm, an exponentially decreasing surviving fraction with dose was observed for these cell lines. An increase in cell inactivation with carbon-ion LET was also seen in the most radiosensitive cell lines (SCC25, HF19). However, the gain in cell inactivation effectiveness appeared of a lower magnitude and with a minor dependence upon increasing LET in the radiosensitive cell lines compared to those that were more resistant to γ-irradiation (Table 2).

Figure 3 shows the SF2 for the various cell lines as a function of LET. The general trend is a decrease in survival following 2 Gy with increasing LET up to 94 keV/μm followed by an increase at higher LET values. The largest variation in monoenergetic carbon-ion effectiveness is observed for SQ20B cells, which are the most resistant to γ-rays (Fig. 2). Their SF2-LET relationship is similar to that found for the radio-resistant Chinese hamster V79 cells. For the other human cell lines, dependence on LET is less pronounced. Interestingly, at the highest LET value (303 keV/μm) the differences in SF2 among the various cell lines are strongly reduced.

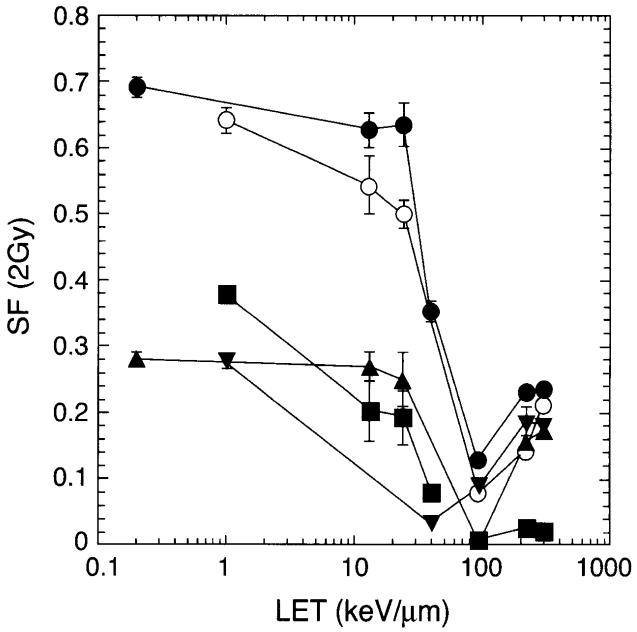

Fig. 3. SF(2 Gy) versus LET for all cell lines and monoenergetic carbon-ions. (●)SQ20B; (■)M10; (▲)SCC25; (▼)HF19; (○)V79.

Cell inactivation along the Spread Out Bragg Peak (SOBP)

M/10, SCC25 and SQ20B human cells, as well as Chinese hamster V79 cells, were irradiated at three different positions along the SOBP of the HIMAC 290 MeV/amu therapeutic carbon beam. The corresponding inactivation curves are shown in Fig. 4. The parameters from the best fits, α and β, together with the SF2 and the RBE values are reported in Table 3. A shouldered dose-response curve was obtained for all cell lines except for the γ-ray radiosensitive SCC25.

As above reported for the exposure to monoenergetic beams, SOBP irradiation also resulted in an increase in cell inactivation with dose-averaged LET, such an LET-dependence of cell inactivation being more pronounced in γ-ray resistant cell lines.

DISCUSSION

Irradiation with monoenergetic carbon-ions

This work intended to investigate the response to carbon-ions of five cell lines, four of human and one of rodent origin, having different sensitivity to γ-rays.

Our data for Chinese hamster V79 cells, which were used as a reference cell line, show that the RBE at 10% survival is consistent with values reported for this parameter by Kanai *et al.*[37] and Furusawa *et al.*[38] for the same cell line irradiated using non-SOBP beams. Likewise, the position of the maximum in our data is consistent with that (around 150–200 keV/μm) reported by the NIRS group.[37,38] The GSI

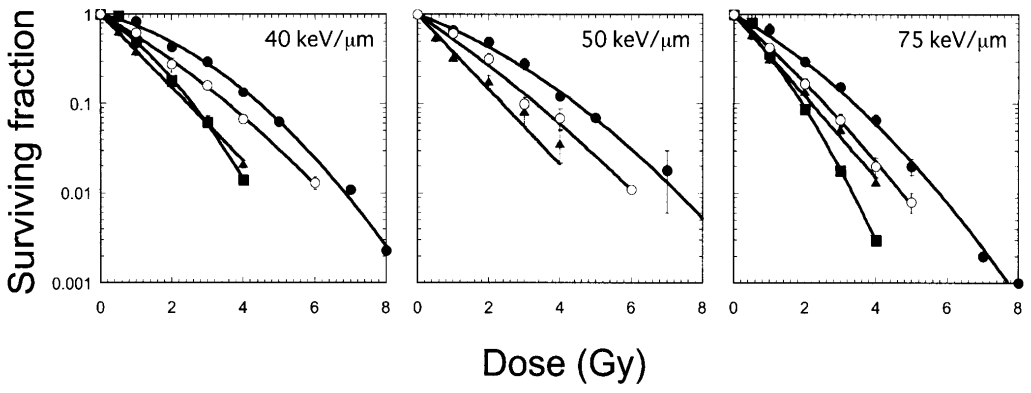

Fig. 4. Cell inactivation by 290 MeV/SOBP carbon-ions. (●)SQ20B; (■)M10; (▲)SCC25; (○)V79.

Table 3. Summary of cell survival parameters and RBE values after irradiation with carbon-ions at different positions along the SOBP

Cell line	LET*(keV/μm)	α (Gy-1)	β (Gy-2)	SF (2 Gy)	RBE (α/αγ)	RBE (2 Gy, γ)	RBE (10%)
Human							
M/10	(γ-rays)	0.30 ± 0.05	0.068 ± 0.009	0.420 ± 0.044			
	40	0.59 ± 0.08	0.118 ± 0.022	0.191 ± 0.033	1.98 ± 0.41	1.69 ± 0.15	1.57 ± 0.17
	75	0.94 ± 0.08	0.129 ± 0.022	0.091 ± 0.016	3.16 ± 0.58	2.42 ± 0.16	2.09 ± 0.20
SCC25	(γ-rays)	0.63 ± 0.02		0.281 ± 0.009			
	40	0.94 ± 0.02		0.152 ± 0.005	1.49 ± 0.05	1.49 ± 0.03	1.49 ± 0.05
	50	0.97 ± 0.05		0.145 ± 0.015	1.52 ± 0.09	1.52 ± 0.08	1.52 ± 0.09
	75	1.05 ± 0.02		0.122 ± 0.004	1.66 ± 0.05	1.66 ± 0.03	1.66 ± 0.05
SQ20B	(γ-rays)	0.16 ± 0.01	0.012 ± 0.001	0.693 ± 0.014			
	40	0.23 ± 0.03	0.065 ± 0.004	0.488 ± 0.029	1.44 ± 0.20	1.67 ± 0.12	1.95 ± 0.10
	50	0.34 ± 0.06	0.039 ± 0.001	0.430 ± 0.056	2.17 ± 0.40	2.08 ± 0.30	1.96 ± 0.25
	75	0.51 ± 0.04	0.050 ± 0.006	0.296 ± 0.020	3.20 ± 0.31	2.96 ± 0.20	2.56 ± 0.14
Rodent							
V79	(γ-rays)	0.18 ± 0.05	0.025 ± 0.011	0.635 ± 0.060			
	40	0.52 ± 0.05	0.034 ± 0.012	0.305 ± 0.035	2.98 ± 0.85	2.44 ± 0.23	1.87 ± 0.24
	50	0.61 ± 0.06	0.023 ± 0.011	0.267 ± 0.036	3.49 ± 1.01	2.78 ± 0.28	2.00 ± 0.27
	75	0.82 ± 0.01	0.032 ± 0.009	0.172 ± 0.008	4.64 ± 1.25	3.66 ± 0.06	2.60 ± 0.25

*Dose-averaged LET (LET_d), evaluated as reported in Materials and Methods.

group also reported a maximum cell-killing in the range of 150–200 keV/μm for Chinese hamster ovary cells.[10] We observed the maximum efficiency for cell inactivation at 94 keV/μm LET for three out of the four human cell lines studied, irrespective of the parameter used for RBE evaluation (Table 2). HF19 cells showed the smallest dependence on LET with the lowest survival level at 39 keV/μm. We continue to find an anomalous behaviour for this cell line, as previously observed by us.[11] The LET-independent cellular response of HF19 foetal fibroblasts is in contrast with the LET-dependent survival curves for irradiated normal human adult fibroblasts like AG01522 and NB1RGB[23,39] and an easy explanation for it cannot be inferred. It can be argued that the LET-independent behaviour might be related to the foetal origin of the HF19 (and HFFF2) cells. In this regards some authors have shown that marked differences exist between foetal and adult fibroblast in terms of proliferation processes. In particular, for example, proliferation of foetal and adult fibroblasts is differentially regulated, in connection with a different action of TGF-beta[40] that seems to be also involved in cellular response to radiation and connected to radiosensitivity.[41]

Nevertheless, it should be noted that, as regards carbon ions, no data have been collected between 39 and 94 keV/μm, and the actual peak could then still be around 80–90 keV/μm.

For the other cell line, the position on the LET axis of the maximum RBE for cell inactivation by carbon-ions around 100 keV/μm agrees with other studies.[10,37]

Our data indicate that the LET interval in which cell inactivation peaks following monoenergetic carbon-ion irradiation is not significantly affected by cell origin, i.e., if the cells derive from normal or tumour tissues, nor does it seem to be influenced by the line's response to low LET irradiation. On the other hand, the maximum RBE depends on the cell line tested: the closed symbols in Fig. 5 depict the RBE (2Gy, γ)-LET relationship for each cell line irradiated with monoenergetic carbon-ions. Such a relationship increases steeply up to 94 keV/μm except for HF19 cells, as already mentioned. At this LET the RBE values range from about 3 to 6 in the four human cell lines. The maximum value, observed for SQ20B cells, is close to the value obtained for

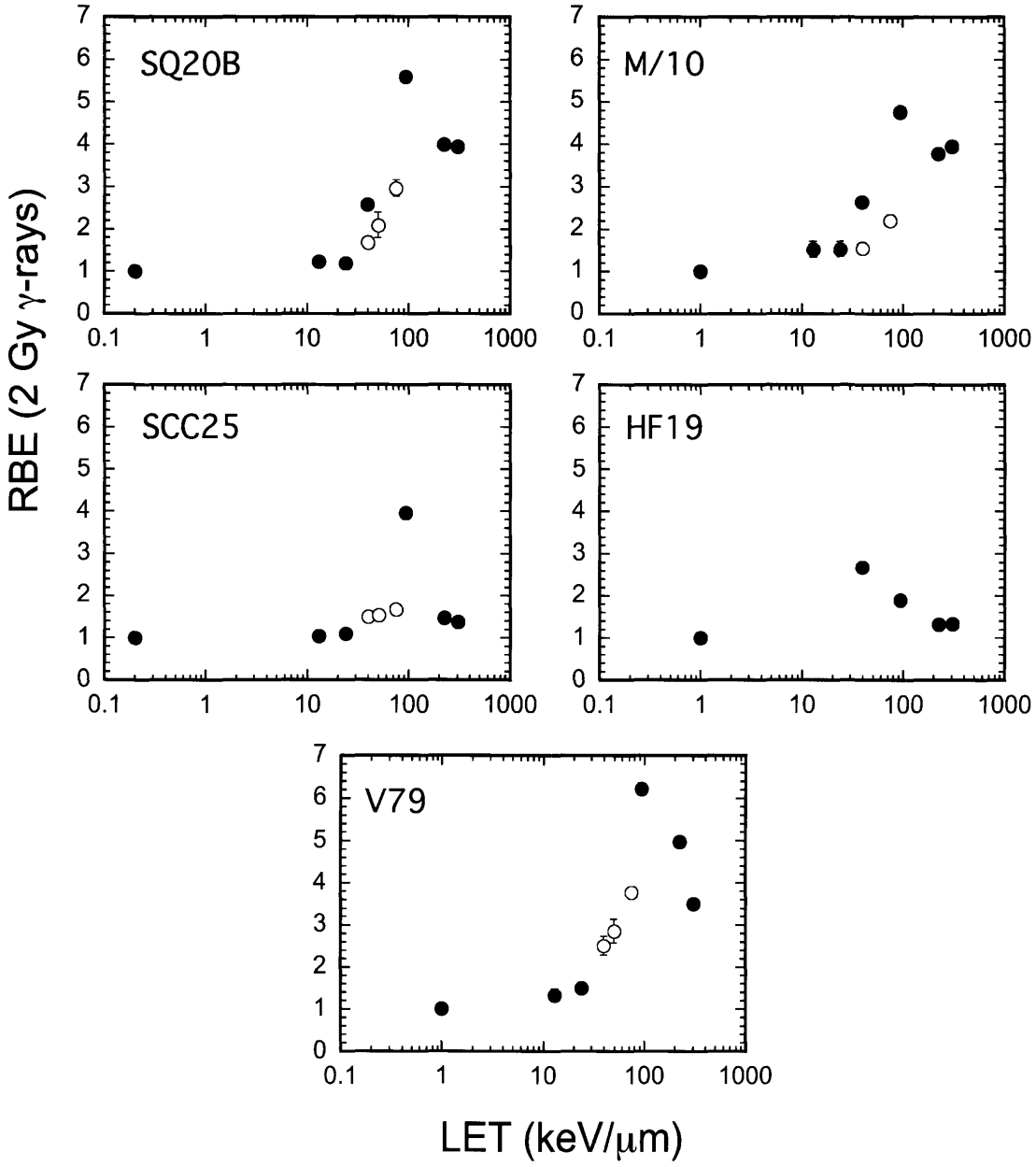

Fig. 5. RBE(2 Gy, γ) vs LET. Closed symbols correspond to monoenergetic beams; open symbols refer to the SOBP irradiation.

V79 cells, suggesting that cells that are more radioresistant to γ-rays show the greatest enhancement in cell inactivation effectiveness.

Radiation sensitivity to γ-rays, as measured through SF2, varies by a factor of ~2.5 among the four human lines. Irradiation of the same cells with high-LET ions produces much less variation in response. Thus, the maximum RBE(2Gy, γ) varies among the four cell lines by approximately the same factor, with higher values tending to be associated with cells possessing higher resistance to γ-rays. Indeed, the origin of the differential radiosensitivities of the squamous cell carcinomas is not known in detail, but it has been recently proposed that it can be related to ceramide-triggered apoptosis pathways and endogenous glutathione levels.[42] In fact, raft coalescence to larger membrane platforms associated with the externalization of an acid sphingomyelinase, leading to ceramide release in raft, is defective in the radioresistant SQ20B cells. Moreover, SQ20B are protected against radiation injury through a fivefold upper level of endogenous glutathione compared to SCC61. Furthermore it has been recently reported[43] that attenuation of the human heat shock protein (Hsp27) in SQ20B cells radiosensitizes the cells giving increased apoptosis, clonogenic cell death and decreased glutathione basal level after photon irradiation. Similar results were found for other tumor radioresistant cell lines overexpressing the Hsp27 protein. In addition a concentration dependent radioprotection leading to significant decrease in apoptotic cells was found in Jurkat-

Hsp27 cells which involved a significant increase in glutathione levels associated with detoxification of reactive oxygen species, delay in mitochondrial collapse and caspase activation.

We also observed a decreased difference in the range of radiation sensitivity variation within the same cells used here in a previous work[11] after irradiation with low-energy protons, the minimum in such difference being at LET≈30 keV/μm. It should be noted that these protons, although of lower LET compared to the carbon-ions used here, are densely ionizing at local scale.

Altogether, these results indicate that cell lines highly responsive to low LET irradiation are greatly affected by radiation quality and generalize the notion, of great practical importance in hadrontherapy, that densely ionizing particles are able to effectively kill cells that are resistant to X- or γ-rays.

Weyrather *et al.*[10] compared Chinese hamster cells of different repair capacity following irradiation to monoenergetic carbon-ions in the 13.7–482.7 keV/μm LET range. They concluded that RBE maximum mainly depended on the α/β ratio, which is thought to reflect cell repair capacity.[44] However, Suzuki *et al.*[15] found that RBE (10%) of 77 keV/μm carbon-ions did not vary significantly among 16 different human cell lines with different α/β ratios for X-rays. In a recent paper,[45] Weyrather and Kraft analyzed the RBE_α dependence in terms of the β/α ratio for rodent and human cells irradiated with three different carbon LET values (13, 77 and 153 keV/μm). The β/α ratio was chosen instead of the α/β ratio because the RBE as a function of β/α does not diverge at very small β, as would be the case for particle irradiation for which the β value is very small. The authors of this study concluded that, for the low LET radiation, the RBE_α values slightly increase with increasing values of the β/α ratio, whereas at the highest LET, the RBE_α strongly increases with increasing β/α, i.e. with the cell repair capacity.

The cell lines used in the present study show a restricted range of β/α values. In fact, for two of them (HF19 and SCC25) β/α is 0, and for the other three lines β/α varies between 0.08 and 0.23. However, our results indicate that for the highest LET values, RBE_α is higher for cells which show a high β/α ratio for γ-rays irradiation, qualitatively agreeing with the result by Weyrather and Kraft.[45]

Irradiation with SOBP carbon-ions

The survival curves from SOBP irradiation confirm a greater effectiveness in cell inactivation compared to γ-irradiation (Table 3 and Fig. 4). In Fig. 5 the RBE values are reported relative to SOBP and monoenergetic beams. It can be seen that SOBP points (these measurements were performed for four out of the five cell lines here studied) do not fit the RBE-LET relationship observed for monoenergetic beams. In all cases, the RBE values for SOBP beams are lower than those relative to monoenergetic beams, such differences depending on the cell line.

It is interesting to directly compare inactivation induced in M/10 and SQ20B cells by monoenergetic beams with SOBP beams at similar LET of 39–40 keV/μm. For both lines, the monoenergetic beam is more effective than SOBP when the dose-averaged LET is considered for such comparison, as shown by the survival curve parameters and by the RBE values. In addition, while a linear function fits the survival data well for M/10 cells following monoenergetic beam irradiation, a linear-quadratic function is required to adequately fit the SOBP data. Moreover, in these cells the 75 keV/μm SOBP beam has a lower effectiveness than the 40 keV/μm monoenergetic beam. Also for SQ20B cells the shape of survival curve changes depending whether they are irradiated with monoenergetic or SOBP beams, mixed fields tending to increase the curvature. This is qualitatively seen by comparing the curves in Figs. 2 and 4, and is quantitatively reflected in the α and β coefficients. After irradiation with a 39 keV/μm monoenergetic beam the ratio $\sqrt{\beta}/\alpha$ (a measures for the curvature) is 0.45, while it increases to 1.2 when irradiation is performed with the 40 keV/μm dose-average SOBP beam. As a consequence, not only the RBE values for the two beams are expected to differ, but this difference will also depend on the level of effect considered.

The difference between the responses to monoenergetic and SOBP carbon-ion beams poses the question whether the dose-averaged LET in a point of a SOBP carbon beam is equivalent, from the radiobiological point of view, to the same LET of a monoenergetic beam. This issue has not been yet addressed in sufficient detail thus far, although it may have a significant impact for establishing the "clinical" RBE.

We conclude that mixed fields as those generated by SOBP beams are less effective than monoenergetic beams of the same dose-averaged LET and therefore their biological effectiveness cannot be evaluated through this parameter. It appears that there is a systematic deviation related to the averaging procedures in the presence of an LET distribution along the SOBP. Moreover, if this distribution is large enough to include high LET values that fall close or beyond the RBE maximum, then the "overkill" effect could result in a further decrease in the biological effectiveness of the beam. Low LET fragments could also play a role. Overall, these results highlight the need of replacing the LET with more suitable descriptors of the radiobiological relevant SOBP characteristics.

The RBE variations with LET, which were smaller for radiosensitive than for radioresistant cell lines following irradiation with monoenergetic beams, were even smaller for SOBP irradiation, i.e. in situations of mixed fields. This observation clearly indicates that those cells that are relatively resistant to X- or γ-rays and whose response varies considerably when more densely ionizing radiation is used, are on the contrary sensitive biological detectors of variations in radiation quality along a SOBP.

CONCLUSIONS

In the panel of normal and tumour cell lines used here, an increase in cell-killing was observed that reflected the increase in the incident LET for both monoenergetic and SOBP irradiation. This is consistent with the induction by carbon-ions of lesions that are less efficiently repairable than after X- or γ-rays in cell lines of varying repair capabilities.

The variation in RBE for cell inactivation was greatest in those cell lines that are more resistant to low LET irradiation, pointing to carbon-ion beams as the radiation of choice for the treatment of tumours resilient to conventional radiotherapy.

The lower effectiveness of SOBP compared to monoenergetic carbon-ion beam irradiation argues against a straightforward radiobiological equivalence between the dose-averaged LET in a point of a carbon-ion SOBP and the monoenergetic beam at the same LET.

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