COMPARISON OF RADIOBIOLOGICAL EFFECTS OF CARBON IONS TO PROTONS ON A RESISTANT HUMAN MELANOMA CELL LINE*

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Abstract

Radiobiological parameters were assessed after irradiation of HTB140 melanoma cells with 62 MeV/u ^{12}C ions or protons along Bragg curve. Due to higher linear energy transfer (LET), better efficiency of ^{12}C ions compared to protons was revealed through surviving fraction and relative biological effectiveness. Significant cell inactivation on distal declining end of Bragg peak was higher for ^{12}C ions. Protons, close to Bragg peak maximum, having lower LET, produce reparable damages to affected cells, thus lower proliferation than ^{12}C ions. Higher LET of ^{12}C ions causes more irreparable damages, giving higher proliferation activity.

INTRODUCTION

Ion beams have been commonly used in radiotherapy because of their good radiobiological response in killing malignant growth. Along the particle track, energy deposited per unit of length, i.e. linear energy transfer (LET), explains induced biological effects to radiation damage. However, LET depends on particle energy, as well as on particle species, heavier particles having higher energy loss per unit length. Moreover, relative biological effectiveness (RBE) depends on LET, but also on ion species and cell types [1, 2].

We have extensively analysed effects of proton irradiation, at the Bragg peak as well as along the plateau of SOBP, including the distal fall-off part of SOBP, on human HTB140 melanoma cells. Although rather high RBE values, with respect to γ-rays, were obtained in all cases, surviving fractions at 2 Gy (SF2) revealed that these cells are among most radioresistant cell lines reported [3, 4]. Being the limit case, due to its position on the wide scale of cellular radiosensitivity, HTB140 cells were exposed to the beam of ^{12}C ions at three positions within the Bragg peak. Radiobiological effects corresponding to these three different LET values were analysed and compared to the effects of protons.

EXPERIMENTAL CONDITIONS

Exponentially growing HTB140 melanoma cells (RPMI 1640 medium, 10 % foetal calf serum, 5% CO_2, 37 ^{0}C), were irradiated close to the Bragg peak maximum and in the middle of the spread out Bragg peak (SOBP) of 62 MeV protons, as well as close to the Bragg peak maximum of 62 MeV/u ^{12}C ions. The beams were produced by the superconducting cyclotron at the CATANA treatment facility, INFN – LNS, Catania. Irradiation positions were obtained by interposing Perspex plates (Polymethyl methacrylate - PMMA) in front of the cell monolayer, thus providing corresponding dose levels (Table 1). Irradiations with reference γ-rays, were performed using ^{60}Co source at the Vinča Institute of Nuclear Sciences in Belgrade. All cell irradiations were carried out in air at room temperature. The values of particle mean energy ($\bar{E}$) and LET with standard deviation, given in Table 1, were obtained by numerical simulations with the GEANT4 code [5, 6].

Table 1: Irradiation position parameters

Irrad. posit.	Perspex (mm)	Dose (%)	$\bar{E}$ (MeV)	LET (keV/μm)
^{12}C - ions				
A	6.0	22.04±1.35	31.80±3.72	7.50±0.63
B	8.8	92.11±4.76	8.97±0.92	22.50±2.84
C	9.0	21.42±6.43	4.77±0.65	35.60±4.31
Protons				
	25.0	90.40±4.00	8.59±0.46	6.90±0.50

Evaluation of cell survival was performed by counting Giemsa stained colonies, 7 days after irradiation, time corresponding to seven cell doubling times. More than 50 cells per colony were scored as a surviving cell. The numbers of viable cells were estimated from two duplicate experiments for each irradiation position.

Cell proliferation was estimated 7 days after irradiation using Cell Proliferation ELISA, BrdU (colorimetric) Kit (Roche, Germany). Assay was performed according to the manufacturer's instructions.

RESULTS AND DISCUSSION

Evaluations of cell survival after irradiations with doses ranging from 2 to 16 Gy were carried out at different positions within the depth dose distribution in Perspex. Three positions were chosen for ^{12}C ions and a position close to the proton Bragg peak maximum, having γ-rays as a reference radiation. To fit survival curves the linear-quadratic model was used: $S = \exp(-\alpha D - \beta D^2)$, where S is surviving fraction after dose D, while α and β are free parameters. Surviving fractions were evaluated from these best fit survival curves and are given in Fig. 1.

Cell survival curve for irradiation position A, which belongs to the plateau region of the ^{12}C ions Bragg curve is flat and almost horizontal showing practically no dose dependence. At the position B, corresponding almost to the maximum of the Bragg peak, the survival curve is dose dependent with a rather good fall. When irradiation was carried out at the distal fall-off part of the Bragg peak, the position C, the survival curve was also dose

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dependent, but with a lower drop than at the position B. Cell survival curves for protons at the position close to the Bragg peak maximum and γ-rays exhibit the typical shoulder with the major decrease, particularly for protons, when doses applied increase. However, ^{12}C ions induce stronger inactivation effect at the position B and even at C, than protons (Fig. 1).

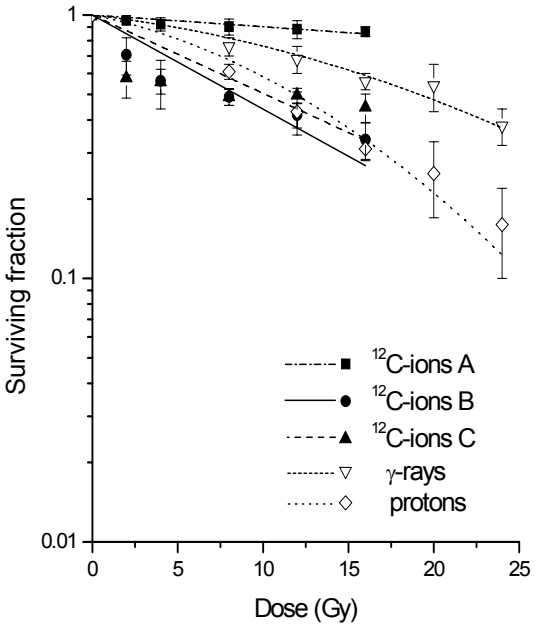

Figure 1: Dose dependent surviving fractions after irradiation with ^{12}C ions (positions A, B and C), protons and γ-rays.

Stronger killing ability of ^{12}C ions is the consequence of their higher LET, while particles mean energy of ^{12}C ions and protons are quite close, as shown in Table 1. Although it has not been considered here in details, it has been shown that, when analyzing biological responses to different ion species, their track structure of the energy deposition, including fragmentation and secondary particles, plays an important role and has to be taken into account. Therefore different biological responses are caused by the different track structure for different atomic numbers with similar LET values [7].

Initial results have indicated very high resistance of HTB140 cells on the wide scale of cellular radiosensitivity [3, 8]. Still, further analyses have been undertaken to investigate this cell line as the potential limit case. Modulating proton energy spread out Bragg peak (SOBP) was obtained and its mid point was also considered and compared to irradiation position B of ^{12}C ions and position close to the proton Bragg peak maximum. All these irradiation positions delivered from 90 (Table 1) to 100 % (SOBP) of dose. Surviving fractions at 2 Gy (SF(2Gy)) are rather high for all irradiations applied, decreasing towards ^{12}C ions, as presented in Table 2. Relative biological effectiveness (RBE), defined as the ratio of 2 Gy γ-ray dose and the proton dose generating the same inactivation level as that given by 2 Gy of the reference γ-rays, obtained from survival curves (Fig. 1), increases towards ^{12}C ions [3]. Better effectiveness at the mid proton SOBP than at the position close to the proton Bragg peak maximum is due to high LET components of superimposed Bragg curves when producing SOBP. Irregular dose dependence of RBE both for ^{12}C ions and mid of proton SOBP is caused by different fitting precision. In spite of such high effectiveness of applied irradiation types, these cells remain highly radioresistant with quite low cell inactivation level.

Effects of ^{12}C ions on cell proliferation, that reflects quality of life of irradiated cells, were assessed as percentage of control values and are not presented here, but some comments are given. In each of the three irradiation positions along Bragg curve dose dependent decrease of cell proliferation capacity was observed. At the position A, a moderate but dose dependent decrease of proliferation was noticed. Moving the irradiation position close to the Bragg peak maximum (position B), BrdU incorporation capacity further decreased. At the distal fall-off (position C) the highest inhibitory effects of irradiation regarding cell proliferation were obtained.

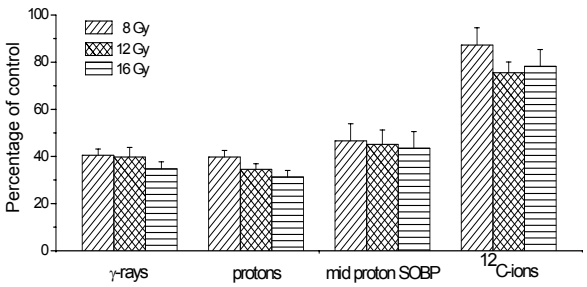

Figure 2: Proliferation capacity estimated after irradiation with ^{12}C ions (position B), protons (close to Bragg peak maximum and mid of SOBP) and γ-rays.

Proliferation capacity of cells after different types of irradiations is given in Fig. 2. It points out that γ-rays and protons close to the Bragg peak maximum produce, apart from irreparable lesions, as shown by corresponding survival curves (Fig. 1), also reparable damage to exposed cells, therefore provoking cell cycle arrest and relatively

Table 2: Radiobiological parameters

Irradiation	SF(2 Gy)	RBE(2 Gy, γ)	RBE(8 Gy, γ)	RBE(12 Gy, γ)	RBE(16 Gy, γ)
γ-rays	0.961±0.004	1	1	1	1
protons	0.931±0.006	1.69±0.17	1.61±0.09	1.64±0.14	1.63±0.13
mid SOBP	0.748±0.103	2.14±0.11	3.16±0.87	3.26±0.79	2.89±0.59
^{12}C-ions	0.706±0.114	3.38±0.64	3.30±0.39	2.80±0.42	2.47±0.34

low DNA duplication [9]. Irradiation in the middle of the proton SOBP induce more irreparable damage, because of the contribution of higher LET components of superimposed Bragg curves of SOBP. Thus, lesser number of reparable lesions allows somewhat higher cell proliferation. Particularly, when cells are irradiated with ^{12}C ions close to the Bragg peak maximum (position B), due to their quite higher LET and therefore lower cell survival (Fig. 1), this effect is even more pronounced. This leads to a rather high level of replication (Fig. 2).

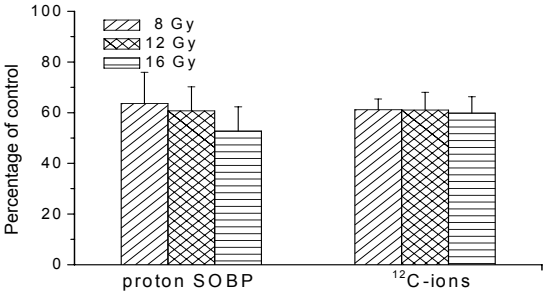

Figure 3: Proliferation capacity estimated after irradiation with ^{12}C ions (position C) and protons (distal declining end of SOBP).

Another important concern is the effect of particles at the distal fall off part of the Bragg curve. This is particularly important for hadron therapy planning when a vital healthy tissue is located just behind the treated tumour. Therefore, cell proliferation capacity at the irradiation positions at the distal declining end of the proton SOBP (where the dose level was 32.12±4.27 %), and of ^{12}C ions (position C) was assessed and is given in Fig. 3. It appears that proliferation capacities are quite close in both cases that imply similar levels of irreparable and reparable lesions.

Considering that the difference in dose levels is about 10 % and that the degrees of cell proliferation are almost equal, one might expect that for the same dose levels ^{12}C ions would, due to their higher LET, produce more irreparable and less reparable damages to the cells affected by irradiation, thus reaching higher proliferation level.

Since radiation dose is proportional to particle fluence and LET, when analysing the distal fall off part of the Bragg curve or SOBP, variation of these parameters should be taken into account [10]. At the energy used in this experiment (62 MeV/u), particle fluence decreases very slowly almost until the end of range, particularly in the case of protons, since it is slightly influenced by secondary particles produced in interactions. Close to the end of range the fluence falls down very fast to zero, while LET rises exponentially, producing the distal declining end of the Bragg curve. Consequently, the decreasing number of particles has leaser hits on irradiated cells. However, these hits by particles with increasing LET cause more irreparable and less reparable lesions. Therefore, there is a relatively higher killing ability of a smaller number of particles when approaching the end of range. This explains why carbon dose dependant survival curves at irradiation positions B and C (Fig. 1) are close although there is an important difference in dose levels (Table 1). The same reasoning can be used to justify also close dose dependant survival curves at the middle and at the distal declining end of SOBP (data not presented). In addition, relatively high values of dose dependant cell proliferation at the distal declining end of proton SOBP and carbon Bragg curve (Fig. 3) are due to a smaller number of hits to irradiated cells and to a larger number of irreparable lesions induced by these hits. Thus a greater number of cells can maintain active proliferation instead of being involved in complex repair processes.

CONCLUSIONS

After irradiations with γ-rays, protons and ^{12}C ions evaluated radiobiological parameters have shown high resistance of human HTB140 melanoma cells. Due to higher LET, ^{12}C ions have caused better effectiveness compared to protons. When receiving maximum dose, cells irradiated with γ-rays or protons, having lower LET, have shown lesser dose dependent proliferation than for ^{12}C ions. This is correlated to different quality of damage produced in irradiated cells. In addition, there is a relatively higher inactivation capacity of protons and ^{12}C ions on the distal declining end of the Bragg curve compared to the Bragg peak. Reported study, dedicated to the limit of cellular radiosensitivity, reveals how far protons and carbon ions could reach in producing biological response.

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