INT. J. RADIAT. BIOL 2000, VOL. 76, NO. 10, 1297–1303

Radiobiological studies on the 65 MeV therapeutic proton beam at Nice using human tumour cells

D. BETTEGA†, P. CALZOLARI†, P. CHAUVEL‡, A. COURDI‡, J. HERAULT‡, N. IBORRA‡, R. MARCHESINI§, P. MASSARIELLO†, G. L. POLI† and L. TALLONE†*

(Received 11 February 2000; accepted 23 May 2000)

Abstract.
Purpose: To determine the relative biological effectiveness (RBE) for initial and delayed inactivation of cells by a modulated proton beam suitable for the treatment of tumours of the eye, within the spread-out Bragg peak and in its distal declining edge.
Materials and methods: Human tumour SCC25 cells were irradiated with the 65 MeV proton beam at the Cyclotron Medicyc in Nice. Perspex plates of different thickness were used to simulate five positions along the beam line: 2 mm corresponding to the entrance beam; 15.6 and 25 mm in the spread-out Bragg peak; 27.2 and 27.8 mm for the distal edge. At each position clonogenic survival of the irradiated cells and of their progeny were determined at various dose values. ^{60}Co γ-rays were used as reference radiation.
Results: RBE values evaluated at the survival level given by 2 Gy of γ-rays increased with increasing depth from close to 1.0 at the proximal to about 1.2 at the distal part of the peak. Within the declining edge it reached the value of about 1.4 at 27.2 and about 2 at 27.8 mm. For the progeny of irradiated cells, the RBE value ranged from 1.0 to 1.1 within the spread-out Bragg peak and then increased up to a value of 2.0 at the last position. The dose–effect curves for the progeny always had a larger shoulder than for the irradiated progenitors, their α parameters being lower by a factor of about 4 and their β parameters always being higher. The α/β ratio was about 50 Gy for the progenitors and about 6 Gy for their progeny. The incidence of delayed effects increased with dose and with the depth within the beam.
Conclusions: RBE values for the inactivation of cells irradiated in the spread-out Bragg peak are compatible with the value currently assumed in clinical applications. In the distal declining edge of the beam, the RBE values increased significantly to an extent that may be of concern when the region of the treatment volume is close to sensitive tissues. The yield of delayed reproductive cell death was significant at each position along the beam line.

1. Introduction

Several tumour types are presently treated with therapeutic proton beams with positive results. As a consequence, interest in the development of hospital-based proton therapy facilities is increasing (Amaldi 1998). The Bragg peak associated with proton beams gives good physical dose distributions; for radiotherapy, the Bragg peak is spread out by modulating the energy of the particles to cover a well-defined target volume at a given depth. In the spread-out Bragg peak (SOBP), particles have a broad spectrum of energy which is dependent on the initial energy. For a given initial energy, the spectrum may vary significantly with the shaping of the beam. The SOBP therefore has a higher LET than the entrance beam. In addition, there is an LET gradient from the proximal to the distal part of the SOBP. The relative biological effectiveness (RBE) of protons varies with LET. Knowledge of the actual RBE value is important both for calculation of the minimal dose necessary for tumour control and also for the determination of the maximal dose to the tumour region when effects on critical normal tissues are the limiting factor.

Determination of RBE at different points in the beam has been done in almost all the radiotherapy centres. Various authors have reported data on modulated proton beams with energy less than 100 MeV that are used for the treatment of eye tumours: Matsubara *et al.* (1990) on the 70 MeV proton beam at Chiba using chromosome aberrations in peripheral lymphocytes; Blomquist *et al.* (1993) on the 72 MeV proton beam at the Uppsala Svedberg laboratory using survival in human colon carcinoma cells, LS174T, and in V79 cells; Courdi *et al.* (1994) on the 65 MeV beam at the Nice Antoine-Lacassagne Centre for survival in the human melanoma cells, CAL4; Gueulette *et al.* (1996) on the 85 MeV proton beam at the Louvain-La-Neuve for survival in CHO cells, and for intestinal crypt regeneration in mice; Wouters *et al.* (1996) on the 70 MeV beam at TRIUMF for survival in V79 cells; and Tang *et al.* (1997) on the 65 MeV beam at the RCNP, Osaka, for survival in CHO cells. Studies on cell survival have been confined to the region before the distal part of the beam. Blomquist *et al.* (1993) and Gueulette *et al.* (1996) reported RBE values between

*Author for correspondence. e-mail: lucia.tallone@mi.infn.it
†Dipartimento di Fisica, Università di Milano, Via Celoria 16, 20133 Milano, Italy.
‡Centre Antoine-Lacassagne, Batiment du Cyclotron, 227 Avenue de la Lanterne, F-06200, France.
§Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milano, Italy.

1.0 and 1.2. In particular, Gueulette *et al.* measured the microdosimetric spectra throughout the SOBP and compared these with a 'biological weighting function' (Pihet *et al.* 1990, Loncoln *et al.* 1994), which gives the RBE as a function of the mean lineal energy, y, for intestinal crypt cell regeneration. The authors showed that the dose–mean lineal energy varied with depth from 5.72 to 8.35 keV/μm. Moreover, the energy deposition events with y higher than 20 keV/μm, for which the RBE is expected to rise sharply, were only a small part of the energy deposition spectra. They concluded that proton RBE values could be high only at the most distal and descending part of the SOBP.

Courdi *et al.* (1994), Wouters *et al.* (1996) and Tang *et al.* (1997) have reported RBE values increasing with depth in the SOBP up to 1.4–1.6. Matsubara *et al.* (1990) have also reported values up to 2.5 based on the frequency of dicentrics in human lymphocytes at a depth of 25 mm from the entrance in a 70 MeV modulated proton beam. Paganetti (1998) calculated the spatial variation of the RBE within the SOBP of the 68 MeV proton beam produced at the HMI–Berlin eye treatment facility. The proton energy distributions at different positions were obtained by Monte-Carlo transport calculations and RBE values were calculated using a track structure model and the radiosensitivity parameters of the CH2B32 and the V79 cell lines. The authors found RBE values that increased with depth up to 1.5 at the end of the SOBP.

The region beyond the distal point, the so-called 'declining distal edge' is less investigated (Egger *et al.* 1997). Several authors, Gueulette *et al.* (1996), Wouters *et al.* (1996), Belli *et al.* (1997) and Paganetti (1998), have suggested that RBE values increase in this region. To the present authors' knowledge, no cell inactivation measurements have been performed in this region for proton beams with energies less than 100 MeV, a clinically important area.

Recently, there has been considerable interest in delayed radiation effects. The reduction in clonogenic potential of the progeny of cells that survive radiation exposure, termed in the literature as delayed reproductive death (DRD), is of importance in radiotherapy. It is generally thought that this effect, transmissible over many generations, could be due to induced genomic instability in the cell population (Seymour *et al.* 1986, Gorgojo and Little 1989, Morgan *et al.* 1996, Limoli *et al.* 1997, Mothersill and Seymour 1997, 1998, Little 1998, Mendonca *et al.* 1998, Wright 1998).

Several studies have been reported on the role of radiation quality for the expression of genomic instability observed as the presence of chromosomal aberrations, apoptosis, or micronucleus formations. Kadhim *et al.* (1992, 1995) observed chromosomal instability in mouse and human haemopoietic stem cells irradiated with α-particles but not with X-rays. A similar LET dependence was observed for the same effect with primary human fibroblasts after exposure to heavy ions and to the X-rays (Martins *et al.* 1993). In other studies, low-LET radiation was proven to be relatively effective when compared with high LET (Little *et al.* 1997, Manti *et al.* 1997, Ponnaiya *et al.* 1997, Trott *et al.* 1998, Ullrich and Ponnaiya 1998). Kadhim *et al.* (1998) reported on two human fibroblast lines (HF19 and HF12) irradiated with X-rays, neutrons or α-particles. They found chromosomal instability with the three radiations in the HF19 cell progeny and no expression in the HF12 cell progeny. Recently, Belyakov *et al.* (1999) reported on delayed damages induced in human fibroblasts irradiated with X-rays and with 110 keV/μm α-particles. These authors found an increased effectiveness of α-particles versus X-rays for micronucleation, apoptosis and loss of clonogenicity. No data, as far as the present authors know, have been reported on DRD induced by ion beams used in radiotherapy.

2. Materials and methods

2.1. *Proton beam irradiation and dosimetry*

At the Cyclotron Medicyc, the Nice Lacassagne Center, 65 MeV protons were produced. Details of the beam geometry, physical characteristics and dosimetry have been reported elsewhere (Courdi *et al.* 1994, Cambria *et al.* 1997). A purpose-built Teflon cylinder, 13 mm in diameter, was used to fit the beam geometry with a Mylar foil, 52 μm thick, on which the cells were plated. Teflon material was chosen for biocompatibility as well as because of its low atomic number and consequently low cross-section. The Teflon cylinder was located in front of the beam, the attached cells occupying an area of about 1.3 cm^2 around the beam axis. Positional precision was of $\pm$ 0.1 mm. The dose variation around the central axis was less than 1%.

Cell samples were positioned at various depths, simulated by interposing Perspex plates of differing thickness. Figure 1 shows the measured axial physical dose profile used in this study. The arrows indicate the five irradiation positions: 2 mm corresponding to the entrance beam; 15.6 and 25 mm in the SOBP; 27.2 and 27.8 mm for the distal declining edge. The corresponding relative doses were 64.5%, 100% and 101.5% with an error of 5% up to a 25 mm depth. The relative doses were 91% and 52% respectively

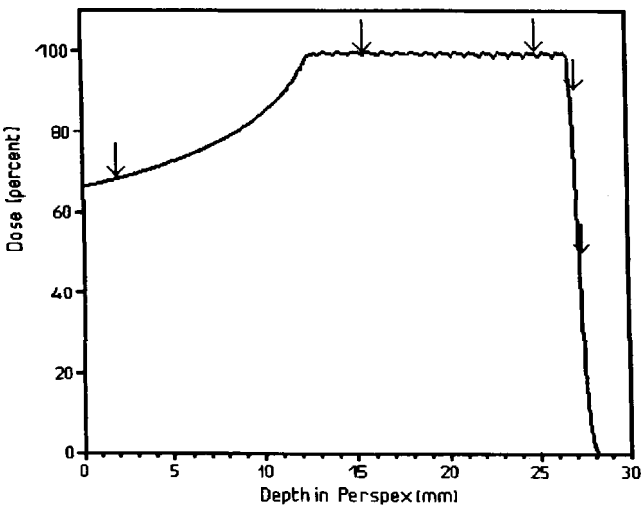

Figure 1. Measured dose in Perspex vs depth for the 65 MeV proton beam produced at the Cyclotron Medicyc at the Nice Antoine Lacassagne Center. Arrows indicate cell irradiation positions.

for the two points in the declining edge and the uncertainty in the dose was estimated to be about 10%. Cells were irradiated at each position at six dose levels from 0.5 Gy to 7 Gy. The average dose rate was 5 Gy/min.

For comparison, cells were irradiated with ^{60}Co γ-rays at the Istituto Tumori di Milano. The dose rate was 1 Gy/min. The maximum build-up was obtained with 0.5 cm of culture medium.

2.2. *Cell culture and survival assay*

The SCC25 cell line, derived from human squamous cell carcinoma of the tongue (Weichselbaum *et al.* 1988), was used. It was kindly provided by Dr E. Blakely with the permission of Dr Weichselbaum. The cells were grown in DME-F12 (75:25) medium supplemented with 0.4 μg/ml hydrocortisone and 20% foetal calf serum. Under these conditions, the plating efficiency was 60% and the doubling time was 24 ± 2 h. The cell thickness, measured by confocal laser microscopy (Sapora *et al.*, in preparation), was 5 ± 1 μm.

Three days before the irradiation, 4×10^4 cells were plated in the irradiation vessel, judged so that at the time of irradiation the cell population would be in exponential growth. At least two samples were irradiated at each dose. The cells were harvested by trypsinization after irradiation, seeded into 25 cm^2 flasks (six flasks per dose) at suitable numbers for inactivation assay and incubated at 37°C. After 18 days of incubation, five flasks for each dose were fixed with methanol and stained with 10% Giemsa solution for evaluation of survival. At the same time one flask for each dose was trypsinized, the cells diluted and seeded at low density into five further flasks and incubated again for 18 days for the delayed reproductive death assay. For both the irradiated cells and their progeny, colonies with more than 50 cells were scored as survivors. The surviving fractions were evaluated by fitting the linear–quadratic model to the data.

3. Results and Discussion

3.1. *Inactivation of the irradiated cells*

Four independent experiments were performed at each position with protons. Three independent experiments were performed with ^{60}Co γ-rays: figure 2 shows the survival data for each of the five proton positions, and results with γ-rays are also shown. The γ-ray curve is very similar to that of the proton beam at 2 mm. The comparison of the survival curves shows that, at the same dose, survival decreased with increasing depth. The decrease was small between 2 mmm and 15.6 mm and larger at 27.8 mm. The data at the 27.8 mm depth appear to deviate from linearity for doses greater than 4 Gy.

Table 1 gives the best fit parameters (α and β) at each position in the proton beam and the

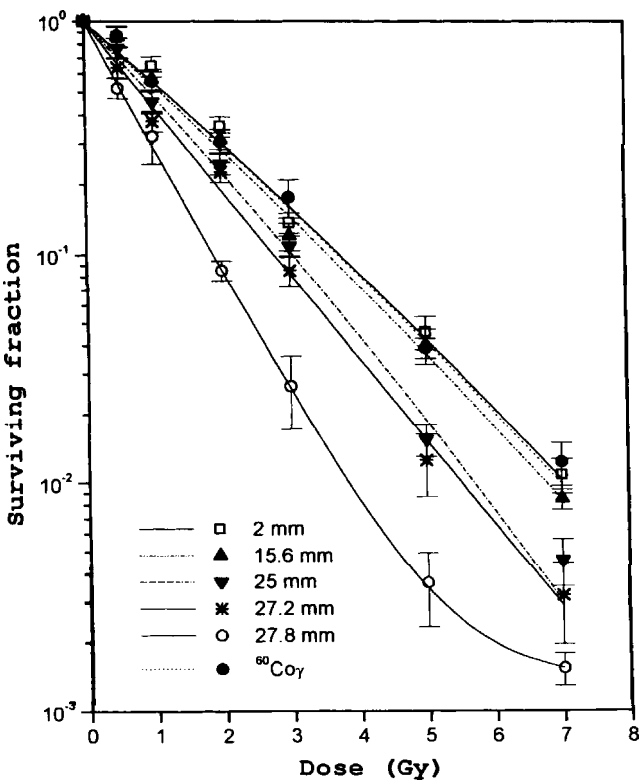

Figure 2. Survival data and fitted curves for the irradiated SCC25 cells at various depths in the proton beam.

Table 1. Summary of survival parameters and relative biological effectiveness (RBE) values.

Radiation	$\alpha(Gy)^{-1}$	$\beta(Gy)^{-2}$	SF(2 Gy)	RBE(2 Gy γ)	RBE(10%)
Irradiated cells					
γ ^{60}Co	0.57 ± 0.06	0.012 ± 0.009	0.30 ± 0.03	1	1
p 2 mm*	0.57 ± 0.06	0.012 ± 0.011	0.30 ± 0.03	0.99 ± 0.13	0.99 ± 0.14
p 15.6 mm	0.61 ± 0.05	0.010 ± 0.008	0.28 ± 0.03	1.05 ± 0.12	1.04 ± 0.12
p 25 mm	0.70 ± 0.06	0.018 ± 0.012	0.23 ± 0.03	1.22 ± 0.14	1.22 ± 0.14
p 27.2 mm	0.83 ± 0.08	0.001 ± 0.014	0.19 ± 0.03	1.39 ± 0.18	1.34 ± 0.19
p 27.8 mm	1.23 ± 0.12		0.09 ± 0.02	2.05 ± 0.27	1.98 ± 0.26
	f = 0.001 ± 0.0001				
Progeny					
γ ^{60}Co	0.17 ± 0.03	0.015 ± 0.006	0.66 ± 0.05	1	1
p 2 mm	0.11 ± 0.01	0.024 ± 0.002	0.73 ± 0.02	0.83 ± 0.12	1.01 ± 0.14
p 15.6 mm	0.19 ± 0.03	0.026 ± 0.004	0.62 ± 0.03	1.13 ± 0.20	1.20 ± 0.18
p 25 mm	0.14 ± 0.01	0.039 ± 0.002	0.64 ± 0.02	1.06 ± 0.15	1.29 ± 0.18
p 27.2 mm	0.15 ± 0.02	0.032 ± 0.004	0.65 ± 0.03	1.05 ± 0.17	1.22 ± 0.18
p 27.8 mm	0.34 ± 0.19	0.15 ± 0.07	0.31 ± 0.02	2.19 ± 0.67	2.25 ± 0.44
	f = 0.05 ± 0.007				

*p—protons.

corresponding RBE values. RBE(2 Gy, γ) indicates the ratio between a 2 Gy γ-ray dose and the equi-effective proton dose. RBE(10%) indicates the ratio of the radiation doses that produce 10% survival. The data at 27.8 mm were analysed, fitted by the equation: $S = f + (1-f)\exp(-\alpha D - \beta D^2)$. The f-parameter is 0.0013 ± 0.0002; it could be interpreted as the fraction of cells that received no dose as many protons come to the end of their tracks near the 27.8 mm depth.

The higher uncertainty in the dose at the two last positions compared with the other three positions (see §2.1) did not affect the reproducibility of the results. Indeed, the standard deviation on α, obtained in four experiments, was 12% compared with values between 4% and 12% at all the other positions. For SF(2 Gy) the standard deviation was 10% and 7% compared with values between 5% and 15% at all other positions. Nevertheless, an uncertainty of 10% in dose induces an uncertainty of about 10% in α and this has been taken into account in the evaluation of the errors reported in table 1 for the two last points. Data in table 1 indicate a higher effectiveness of the proton beam in the region beyond the SOBP compared with the entrance. RBE(2 Gy, γ) values are between 1.0 and 1.2 at up to 25 mm in depth and then reach 1.4 at 27.2 mm and 2.0 at the last position of 27.8 mm.

Figure 3 shows the surviving fraction as a function of depth for 2, 5 and 7 Gy given at 15.6 mm from the entrance. For each depth the dose values were evaluated from the physical dose profile reported in figure 1. Surviving fractions were calculated from the best-fit survival curves at the specified depth. Survival

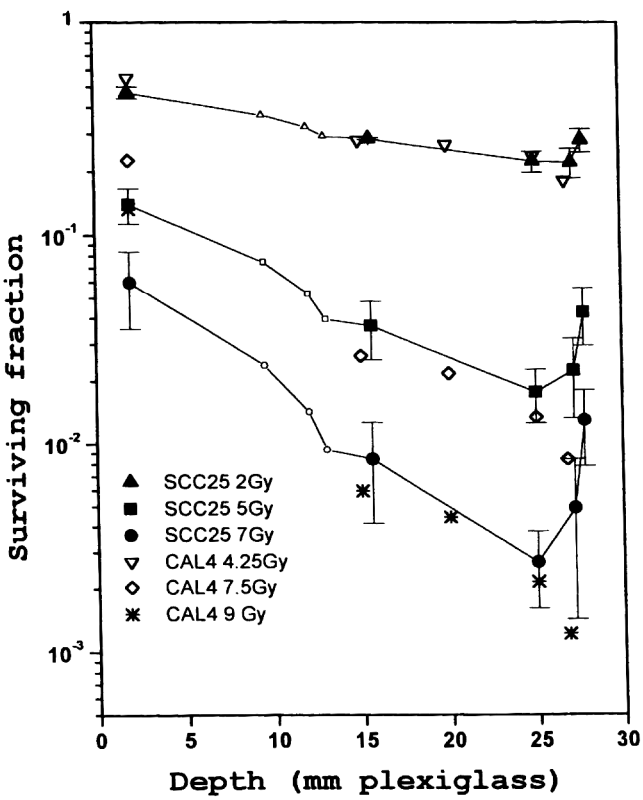

Figure 3. Surviving fraction of SCC25 cells vs depth for doses at 15.6 mm of 2, 5 and 7 Gy. The surviving fractions at 9.5, 12 and 13 mm were calculated using the α and β parameters relative to the 15.6 mm survival curve (small open symbols). Data on the CAL4 melanoma cell line, reported by Courdi *et al.* (1994), for the same beam are also shown for comparison.

decreased with depth ranging from 2 mm to 15 mm following the increase of the physical dose (40%); and from 15 mm to 25 mm following the increase of both the physical dose (1.5%) and the RBE (20%). In the declining distal edge, at 27.2 mm and 27.8 mm depth, the resulting killing effect was comparable with the killing effect at 15.6 mm in the SOBP, although the physical dose in this region was lower (0.90 and 0.50) than in the rest of the SOBP. This is due to the increased biological effectiveness of the radiation. Cell kiiling is therefore extended by approximately 1 mm beyond the distal point of the SOBP (26.8 mm, see the beam profile in figure 1). This extension of the cell killing effect could be of some importance in clinical application.

In figure 3, for comparison, the values calculated from the data published by Courdy *et al.* (1994) on the CAL4 human melanoma cell line irradiated with the same beam (between 2 mm and 26.8 mm) are reported. Doses were chosen to give survival levels at 15 mm depth, which are similar to those of the SCC25. Cell survival decreased from the beginning to the end of the SOBP for the CAL4 cells also. The decrease is particularly evident at 26.8 mm, the distal point of the SOBP.

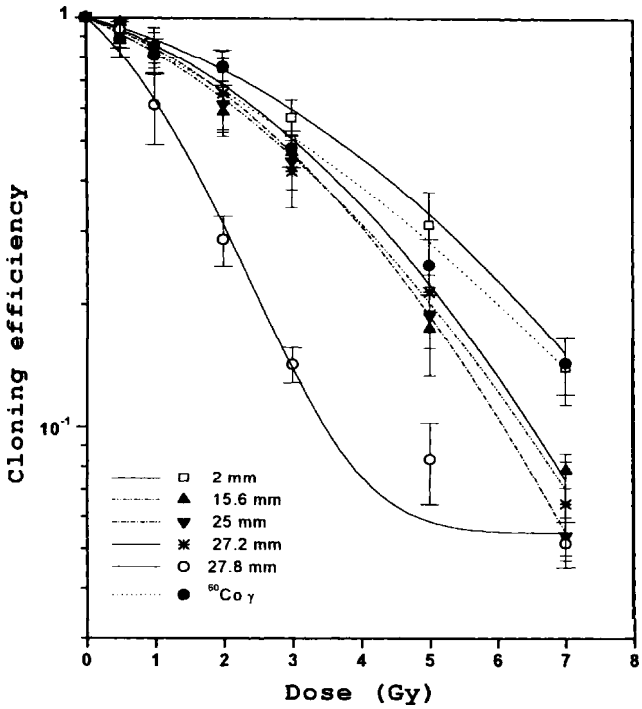

Figure 4. Cloning efficiencies and fitted curves for the progeny of SCC25 cells irradiated with protons vs doses given to their progenitors at various depths in the proton beam.

3.2. *Delayed reproductive death*

Three independent experiments were performed on the progeny of irradiated SCC25 cells at each position for six values of dose given to their progenitors; three similar experiments were done with ^{60}Co γ-rays. The cloning efficiency for each sample was evaluated as the percentage of its own control, that is of the progeny and not of irradiated cells. Figure 4 shows the cloning efficiency data of the progeny and the best fitted curves against the dose given to their progenitors. The cloning efficiency was always lower than 1, a clear sign of the presence of delayed damage in the form of delayed reproductive death. The yield of the effect was significant at all beam positions. There was a slight increase in the effect from the entrance to the proximal point of the SOBP, followed by a constant value and then by a large increase at the last position at which values of RBE equal to 2 were found (see table 1). The data presented provide evidence that both γ-rays and protons can induce genomic instability in SCC-25 cells, visible as delayed reproductive cell death which persisted for several population doublings post-irradiation and increasing with LET.

As can be seen by comparing figures 2 and 4, the cloning efficiency curves of the progeny show greater shoulders than the survival curve of the progenitors. Their α parameters (see table 1) are lower by a factor of 4 and their β parameters are always higher. The α/β ratio is about 50 Gy (for protons between 2 mm and 25 mm and for γ-rays) for the progenitors and about 6 Gy for the progeny. The same results can be obtained from the data reported on human fibroblast by Belyakov *et al.* (1999). The α/β ratios evaluated from the reported parameter values are 28, 3.6 and 5.1 Gy at 0, 8 and 14 days after X-irradiation. With α-particles the survival curve at zero time after irradiation was exponential but at delayed times the curves were shouldered with α/β values of 4.6 and 2.7 Gy. A decrease in the α/β ratio for delayed lethality seems to be a common feature for low- and high-LET radiations. These results seem to indicate that different mechanisms could play a role in the initial and delayed responses. Clutton *et al.* (1996) suggested that a persistent increase in oxi-radical generation occurs in the progeny of the irradiated cells leading to persistent oxidative damage and consequent increase in cell death, mutation and chromosome aberration.

4. Conclusion

The present findings on 65 MeV proton beams indicate that RBE values for cell survival in the spread-out Bragg peak are compatible with the value

of 1.1–1.2, as currently assumed in clinical applications; in the distal declining edge, values as high as 2 are found. The level of cell killing is still comparable with the cell killing at the beginning of the SOBP for about 1 mm beyond the distal point, since the dose reduction is compensated by the increased radiation RBE. This effect is of practical significance in those applications that require exact positioning of the end of the beam. Studies on the progeny of the irradiated cells show the presence of delayed reproductive cell death, which increases with the dose given to the progenitors and with the radiation LET. The shapes of the dose–effect curves for progeny are different from those for the progenitors. This could be an indication that different mechanisms are playing a role in initial and delayed cell damage.

Acknowledgements

This work was supported by the Istituto Superiore di Sanità Italy TOP Project. The authors would like to thank Professor M. Belli for his continuous encouragement and interest.

References

AMALDI, U., 1998, Hadrontherapy in the world and the programmes of TERA foundation. *Tumori*, **84,** 188–199.

BELLI, M., CAMPA, A. and ERMOLLI, I., 1997, A semi-empirical approach to the evaluation of the relative biological effectiveness of therapeutic proton beams. The methodological framework. *Radiation Research*, **148,** 592–598.

BELYAKOV, O. V., PRISE, K. M., TROTT, K. R. and MICHAEL, B. D., 1999, Delayed lethality, apoptosis and micronucleus formation in human fibroblasts irradiated with X-rays or α-particles. *International Journal of Radiation Biology*, **75,** 985—993.

BLOMQUIST, E., RUSSEL, K. R., STENERLOW, B., MONTELIUS, A., GRUSSEL, E. and CARLSSON, J., 1993, Relative biological effectiveness of intermediate energy protons. Comparison with ^{60}Co gamma-radiation using two cell lines. *Radiotherapy and Oncology*, **28,** 44–51.

CAMBRIA, R., HERAULT, J., SILARI, M. and CHAVELT, P., 1997, Proton beam dosimetry: a comparison between the Faraday cup and an ionization chamber. *Physics in Medicine and Biology*, **42,** 1185–1196.

CLUTTON, S. M., TOWNSEND, K. M. S., WALKER, C., ANSELL, J. D. and WRIGHT, E. G., 1996, Radiation-induced genomic instability and persisting oxidative stress in primary bone marrow cultures. *Carcinogenesis*, **17,** 1633–1639.

COURDI, A., BRASSART, N., HERAULT, J. and CHAUVEL, P., 1994, The depth-dependent radiation response of human melanoma cells exposed to 65 MeV protons. *British Journal of Radiology*, **67,** 800–804.

EGGER, E., ZOGRAFOS, L., MUNKEL, G., BOHRINGER, T., BERCHER, L. and CHAMOT, L., 1997, Results of proton radiotherapy for uveal melanomas. *Frontiers of Radiation Therapy and Oncology*, **30,** 111–123.

GORGOJO, L. and LITTLE, J., B., 1989, Expression of lethal mutations in progeny of irradiated mammalian cells. *International Journal of Radiation Biology*, **55,** 619—630.

GUEULETTE, J., GREGOIRE, V., OCTAVE-PRIGNOT, M. and WAMBERSIE, A., 1996, Measurements of radiobiological effectiveness in the 85 MeV proton beam produced at the cyclotron CYCLONE of Louvain-La Neuve, Belgium. *Radiation Research*, **145,** 70–74.

KADHIM, M. A., LORIMORE, S. A., TOWNSEND, K. M., GOODHEAD, D. T., BUCKLE, V. J. and WRIGHT, E. G., 1995, Radiation-induced genomic instability: delayed cytogenetic aberrations and apoptosis in primary human bone marrow cells. *International Journal of Radiation Biology*, **67,** 287—293.

KADHIM, M. A., MACDONALD, D. A., GOODHEAD, D. T., LORIMORE, S. A., MARSDEN, S. J. and WRIGHT, E. G., 1992, Transmission of chromosomal instability after plutonium alpha-particle irradiation. *Nature*, **355,** 738–740.

KADHIM, M. A., MARSDEN, S. J. and WRIGHT, E. G., 1998, Radiation-induced chromosomal instability in human fibroblasts: temporal effects and the influence of radiation quality. *International Journal of Radiation Biology*, **73,** 143—148.

LIMOLI, C. L., KAPLAN, M. I., CORCORAN, J., MEYERS, M., BOOTHMAN, D. A. and MORGAN, W. F., 1997, Chromosomal instability and its relationship to other end points of genomic instability. *Cancer Research*, **57,** 5557–5563.

LITTLE, J. B., 1998, Radiation-induced genomic instability. *International Journal of Radiation Biology*, **74,** 663—671.

LITTLE, J. B., NAGASAWA, H., PFENNING, T. and VETROVS, H., 1997, Radiation-induced genomic instability. Delayed mutagenic and cytogenetic effects of X rays and alpha particles. *Radiation Research*, **184,** 299–307.

LONCOL, T., COSGROVE, V., DENIS, J. M., GUEULETTE, J., MAZAL, A., MENZEL, H. G., PIHET, P. and SABATTIER, R., 1994, Radiobiological effectiveness of radiation beams with broad LET spectra: microdosimetric analysis using biological weighting functions. *Radiation Protection Dosimetry*, **52,** 347–352.

MANTI, L., JAMALI, M., PRISE, K. M., MICHAEL, B. D. and TROTT, K. R., 1997, Genomic instability in Chinese hamster cells after exposure to X rays or alpha particles of different mean linear energy transfer. *Radiation Research*, **147,** 22–28.

MARTINS, M. B., SABATIER, L., RICOUL, M., PINTON, A. and DUTRILLAUX, B., 1993, Specific chromosome instability induced by heavy ions: a step toward transformation of human fibroblasts? *Mutation Research*, **285,** 229–237.

MATSUBARA, S., OHARA, H., HIRAOKA, T., KOIKE, S., ANDO, K., YAMAGUCHI, H., KUWABARA, Y., HOSHINA, M. and SUZUKI, S., 1990, Chromosome aberration frequencies produced by a 70-MeV proton beam. *Radiation Research*, **123,** 182–191.

MENDONCA, M. S., TEMPLES, T. M., FARRINGTON, D. L. and BLOCH, C., 1998, Evidence for a role of delayed death and genomic instability in radiation-induced neoplastic transformation of human hybrid cells. *International Journal of Radiation Biology*, **74,** 755—764.

MORGAN, W. F., DAY, J. P., KAPLAN, M. I., MCGHEE, E. M. and LIMOLI, C. L., 1996, Genomic instability induced by ionizing radiation. *Radiation Research*, **146,** 247–258.

MOTHERSILL, C. and SEYMOUR, C. B., 1997, Lethal mutations and genomic instability. *International Journal of Radiation Biology*, **71,** 751—758.

MOTHERSILL, C. and SEYMOUR, C. B., 1998, Mechanisms and implications of genomic instability and other delayed effects of ionizing radiation exposure. *Mutagenesis*, **13,** 421–426.

PAGANETTI, H., 1998, Calculation of the spatial variation of relative biological effectiveness in a therapeutic proton field for eye treatment. *Phys. Med. Biol.*, **43,** 2147–2157.

PIHET, P., MENZEL, H. G., SCHMID, R., BEAUDUIN, M. and WAMBERSIE, A., 1990, Biological weighting function for RBE specification of neutron therapy beams. Intercomparison of 9 European centres. *Radiation Protection Dosimetry*, **31,** 437–442.

PONNAIYA, B., CORNFORTH, M. N. and ULLRICH, R. L., 1997, Induction of chromosomal instability in human mammary cells by neutrons and gamma rays. *Radiation Research*, **147,** 288–294.

SEYMOUR, C. B., MOTHERSILL, C. and ALPER, T., 1986, High yields of lethal mutations in somatic mammalian cells that survive ionizing radiation. *International Journal of Radiation Biology*, **50,** 167—179.

TANG, J. T., INOUE, T., YAMAZAKI, H., FUKUSHIMA, S., FOURNIER-BIDOZ, N., KOIZUMI, M., OZEKJ, S. and HATANAKA, K., 1997, Comparison of radiobiological effective depths in 65-MeV modulated proton beams. *British Journal of Cancer*, **76,** 220–225.

TROTT, K. R., JAMALI, M., MANTI, L. and TEIBE, A., 1998, Manifestations and mechanisms of radiation-induced genomic instability in V79 chinese hamster cells. *International Journal of Radiation Biology*, **74,** 787—791.

ULLRICH, R. L. and PONNAIYA, B., 1998, Radiation-induced instability and its relation to radiation carcinogenesis. *International Journal of Radiation Biology*, **74,** 747—754.

WEICHSELBAUM, R. R., BECKETT, M. A., SCHWARTZ, J. L. and DRITSCHILO, A., 1988, Radioresistant tumour cells are present in the head and neck carcinomas that recur after radiotherapy. *International Journal of Radiation Oncology, Biology, Physics*, **15,** 575–579.

WOUTERS, B. G., LAM, G. K. Y., OELFKE, U., GARDEY, K., DURAND, R. E. and SKARSGARD, L. D., 1996, Measurements of relative biological effectiveness of the 70 MeV proton beam at TRIUMF using Chinese hamster V79 cells and the high-precision cell sorter assay. *Radiation Research*, **146,** 159–170.

WRIGHT, E. G., 1998, Radiation-induced genomic instability in haemopoietic cells. *International Journal of Radiation Biology*, **74,** 681—687.