Increased Proton Relative Biological Effectiveness at the Very End of a Spread-Out Bragg Peak for Jejunum Irradiated Ex Vivo

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Abstract

Purpose: Currently, it is unclear if the variation in proton relative biological effectiveness (RBE) at different positions in the spread-out Bragg peak (SOBP) should be taken into account for medical applications. To date, the majority of studies have examined cells irradiated in vitro. In this work RBE estimates have been made for intact tissue irradiated ex vivo.

Materials and Methods: Irradiations were performed in the 200 MeV clinical proton beam produced at iThemba LABS (Cape Town, South Africa). The beam was modulated to produce either a 3-cm or a 7-cm SOBP. The number of intestinal crypts regenerated in jejunum sections of mice after single doses of protons was used as the biological end point. For this, a novel ex vivo irradiation technique was implemented to allow measurements at the very end of the SOBP where most variations in proton RBE can be expected.

Results: The proton RBE at the very end of the 7-cm SOBP increases by 10% compared with the middle of the SOBP; the same 10% increase in RBE is observed between the end and the middle of the 3-cm SOBP. This corresponds to an increase of the proton RBE with reference to cobalt-60 gamma rays from 1.14 at the middle of the 7-cm SOBP to 1.25 at the end.

Conclusion: The similarity of the RBE increment for the different SOBP's indicates that most of the increase in RBE occurs over the last 15 mm upstream of the fall-off of the beam. Because the corresponding 10% variation of the biologically effective dose exceeds the dose accuracy standards of 3.5% required in radiation therapy, it is highly advisable to allow for it when designing treatment plans.

Keywords: proton RBE; end of the proton SOBP; Intestinal crypt regeneration in mice; biological treatment plan

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Introduction

To date most proton relative biological effectiveness (RBE) studies have been conducted using cellular systems irradiated in vitro. This approach allows accurate positioning of the samples in a spread-out Bragg peak (SOBP) and hence, sound information about the relationship between RBE and position in a Bragg peak could be obtained. Data for cellular systems treated in vitro do not necessary have the biological relevance required

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for clinical decision making [1]. In addition, as pointed out by Paganetti et al [2], the data from the literature are particularly scattered. The reasons for this include uncertainties in the experimental design and variations in the radiosensitivity of the cell lines used in these studies.

Therefore, the radiobiological program at iThemba LABS focused on using in vivo systems for systematic studies of RBE variations with depth for single and fractionated irradiations [3–5]. In these, 2 biological systems were used: (1) intestinal crypt regeneration in mice that reflects early tolerance of normal tissues and (2) LD_{50} (lethal dose 50%) estimates for mice determined at 6 months after selective irradiation of the thorax. This reflects late tolerance of normal tissues. Both biological end points and irradiation protocols showed an increase in proton RBE with depth in an SOBP. On average, the increase in proton RBE was about 3% from the entrance plateau to the beginning of the SOBP. At positions more distal in the SOBP, the change in RBE was more pronounced and estimated to be about 7%.

The volume of target tissue—either intestine or lung—that was irradiated in these experiments is relatively large, and thus, it was not possible to estimate RBE variations in the last few millimeters of the SOBP. For these reasons a novel technique was developed for irradiating small volumes of intestine. In this, a section of jejenum is externalized and positioned in a plane perpendicular to the axis of the beam. This makes it possible to irradiate small segments of intestine that are positioned as close as 2–3 mm from the very distal edge of the SOBP. With this method, the increase in RBE for the 200 MeV clinical proton beam at iThemba LABS was investigated for crypt regeneration in mice at the very end of a 3-cm and a 7-cm SOBP. The project was approved by the Ethical Committee of the University of Stellenbosch (Project number P07/08/018).

Materials and Methods

Biological System: Intestinal Crypt Regeneration in Mice

This in vivo system that quantifies early response was introduced by Withers and Elkind [6]. It is well described and has numerous advantages, including being independent from environmental conditions and producing steep dose-response curves. This makes the end point particularly suitable for radiobiological intercomparisons. Crypt regeneration was used to intercompare most of the clinical hadron beams worldwide and served as a model for the evaluation of the early tolerance of normal tissue. However, similar to other in vivo systems, relatively high doses must be applied to ensure reasonable sensitivity. RBE studies with low doses given in fractionated irradiation regimens were successfully performed earlier for fast neutrons [7] and protons [4]. The biological rationale and the procedure to quantify crypt regeneration has been extensively documented [8].

In this work, 13-week-old female Balb/c mice bred in the animal house of the University of Cape Town were used. Animals were provided with food and water ad libitum during the entire duration of the experiment.

The 200-MeV clinical proton beam is produced at iThemba LABS using a separated-sector cyclotron. The beam has a fixed horizontal direction and is laterally spread and flattened using a double-scatter/occluding-ring system. The maximum field diameter is 10 cm. The distal 90% to 10% fall-off occurs in 5.5 mm, and the 90% to 10% penumbra of the central axis profiles is 2.8 mm. The Bragg peak is modulated using a rotating stepper-absorber.

Ex vivo irradiations require the use of a restraining device that secures an intestinal loop of jejunum in a well-defined position while maintaining the physiological environment. The device used in this study was made of 3 separate pieces of Lucite that can be assembled together to form a squared target volume that can be accurately positioned in a proton field (**Figure 1**).

Mice were anesthetized using an intraperitoneal injection of ketamine (100 mg/kg) and xylasine (10 mg/kg). The anesthesia lasted between 30 and 45 minutes, which allowed for the irradiation and surgical procedures to be completed. A small portion of the intestine close to the pylorus was externalized and placed in a cavity filled with saline. After the irradiations the intestine loop was placed back in the abdominal cavity, and the surgical cut was closed using stitches.

Whole-body irradiations were administered with the liquid-layer containing the intestine loop positioned perpendicularly to the beam axis. Two separate experiments were performed on different occasions for a 7-cm and a 3-cm SOBP. For the experiment with a 7-cm SOBP, the midplane of the intestine-containing layer was placed either 35 mm or 1.5 mm upstream from the end of the SOBP. These positions are referred to as the middle and end of the 7-cm SOBP. For the 3-cm SOBP, the target volume was placed either at 15 mm, 7.5 mm, or 1.5 mm upstream from the end of the SOBP. These positions are referenced as middle, intermediate position, and end of the 3-cm SOBP.

At 3.5 days after irradiation the mice were euthanized and the irradiated jejunal section removed. The samples were immediately fixed in a Bouin-Hollande solution [9]. The piece of jejunum was embedded in paraffin for each sample, and transversely sliced sections of about 4 μm were stained using the classical trichrome technique.

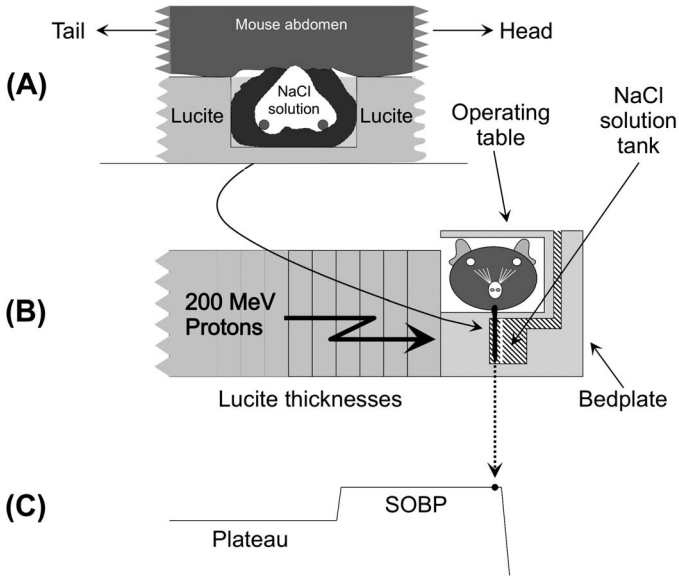

Figure 1. Setup used for irradiating intestine. (A) Beam's eye view: externalized intestinal loop soaked into a 2.5-mm thick gap of NaCl solution. (B) Side view: setup profile showing the NaCl solution tank and the Lucite trellis (vertical white line) limiting the position of the intestine. Varying the Lucite thickness upstream makes it possible to irradiate intestine sections at well-defined positions in the depth-dose profile. (C) Dose profile: in this setup the target volume (closed point on the SOBP) extends in depth only over approximately 2.5 mm (ie, the diameter of the intestine) instead of the 15-mm thickness of the bowel in the case of total body irradiation. That makes it possible to determine RBE values close to the end of the SOBP. Abbreviations: RBE, relative biological effectiveness; SOBP, spread-out Bragg peak.

Results

The dose-effect relations for intestinal crypt regeneration in mice after irradiations at different depths in the SOBP for the 200 MeV clinical proton beam are presented in **Figure 2A** and **2B**. The figures show independent experiments carried out for proton beams modulated to produce a 7-cm or a 3-cm SOBP, respectively. For both experiments, each data point corresponds to the mean number of regenerated crypts observed per circumference in tissue sections obtained from 4 mice. The data points are fitted in log-linear coordinates to straight lines parallel to each other. However, the closed grey and dotted symbols on the figure correspond to data that were not included in the curve fitting. The assumption that a single regenerated crypt corresponds to a single surviving stem cell is not valid for regenerated crypts higher than 70 to 80 per circumference [8], as these data do not belong to the exponential distal part of the cell survival curve. The slopes of the curves expressed in term of D_0 values [10] are equal to 1.35 ± 0.04 Gy in **Figure 2A** and 1.74 ± 0.05 Gy in **Figure 2B**.

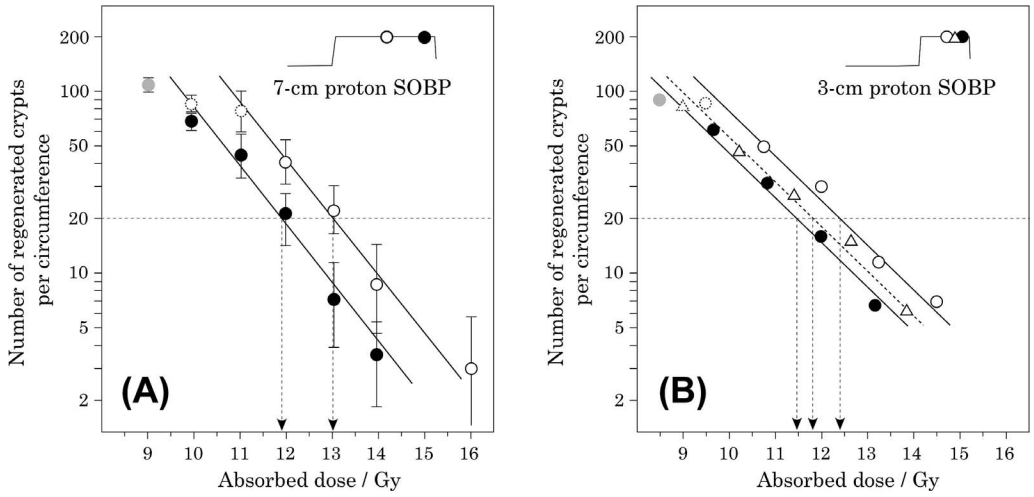

Figure 2. Dose-effect relationships for crypt regeneration in mice after irradiation in a single fraction in the 200 MeV energy-modulated proton beam at iThemba LABS. (A) and (B) correspond to independent experiments performed in beams modulated to produce a 7-cm or a 3-cm SOBP, respectively. The closed circles, open triangles, and open circles correspond to proton irradiations at the end, halfway between the end and the middle, and at the middle of the SOBPs, respectively. The closed grey and dotted symbols correspond to data that were not taken into account for fitting the lines. Each point is the average of the readings observed in 4 mice. Parallel exponential regression curves were fitted through the points by a weighted least squares method. The error bars in (A) correspond to 95% confidence intervals. The error bars in (B) are of the same magnitude but have been omitted for clarity. Abbreviation: SOBP, spread-out Bragg peak.

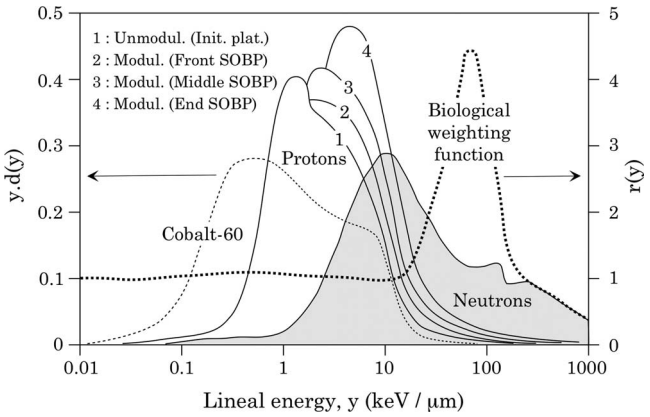

Figure 3. Microdosimetric spectra for a 90 MeV energy-modulated proton beam [17]. Measurements at 4 positions are shown. This is compared with cobalt-60 gamma rays and fast neutrons, dotted line and shaded area, respectively. The BWF for different LET values is also shown. Abbreviations: BWF, biological weighing function; LET, linear energy transfer.

Figure 2A shows the radiation doses corresponding to an isoeffect of 20 regenerated crypts per circumference. These are 13.0 Gy and 11.8 Gy for irradiations in the middle and at the end of the 7-cm SOBP, respectively. These values correspond to an RBE increase of 10% ± 4% from the middle to the end of the SOBP. **Figure 2B** shows that the doses corresponding to the same level of isoeffect are 12.4 Gy, 11.8 Gy, and 11.4 Gy for irradiations in the middle, the intermediate position, and the end of the 3-cm SOBP, respectively. These values correspond to an RBE increase of 5% ± 3% from the middle to the intermediate position of the SOBP and an RBE increase of 9% ± 4% from the middle to the end of the SOBP.

The clinical relevance of these data is better appreciated when RBE values are also expressed relative to photons. To minimize the number of animals used, no direct measurement of the proton RBE with reference to gamma rays was made for ex vivo irradiations. However, we compared the response to gamma rays for ex vivo irradiations with irradiation of the mice to the whole-body or selective irradiations of the bowel through a randomized assay. All 3 techniques yielded similar results, and no statistically significant difference could be established between the doses needed for the regeneration of 20 to 50 crypts per circumference.

Also, we measured proton RBE relative to cobalt-60 gamma rays at the middle of the 7-cm SOBP 3 times previously at iThemba LABS using whole-body irradiations. These experiments yielded RBE values of 1.14 ± 0.03 [3], 1.12 ± 0.03 [5], and 1.15 ± 0.04 [4]. It is appropriate to use the mean of these values, that is, 1.14, to normalize the present ex vivo data to proton RBE values relative to gamma rays. Doing so, RBE values of 1.14 are estimated for the middle of the 7-cm SOBP and 1.25 for the very end of the SOBP.

Discussion

The basic physical information from which predictions about the response of living tissue to radiation can be made are provided by track-structure analysis [11–13] and/or microdosimetric measurements [14, 15]. The fact that selected physical parameters can be related to biological response resulted in biophysical models that can be used to infer the therapeutic outcome of irradiations.

Microdosimetric considerations were used to predict RBE variations as a function of linear energy transfer (LET) [16]. An example of this is given in **Figure 3** [17]. To interpret the microdosimetric spectra shown in the figure and to predict possible RBE variations, readings need to be related to suitable biological weighting functions (BWFs). This allows the RBE to be inferred for different LET values covered by the spectra. The BWF for intestinal tolerance in mice that we previously derived from RBE measurements of different neutrons energies [17] has been superimposed on the spectra. From this the following is noted. First, only a small proportion of the area covered by the proton microdosimetric spectra is associated with the raising part of the BWF. For this, LET values are approximately between 20 and 200 keV/μm. As a consequence, the proportion of the single-energy deposition events that have an RBE larger than unity is very small, which explains why proton beams exhibit low RBE values between 1.10 and 1.20 [18]. By contrast, a larger portion of the microdosimetric spectra for neutron beams is associated with the raising part of the BWF, which explains the higher RBE values of 2 to 4 noted for neutrons [19]). Second, proton microdosimetric spectra are shifting toward high LET values when measured more distally in the SOBP. Consequently, there is an increase in the portion of the spectra associated with the raising part of the BWF. This suggests that the proton RBE would also increase with depth.

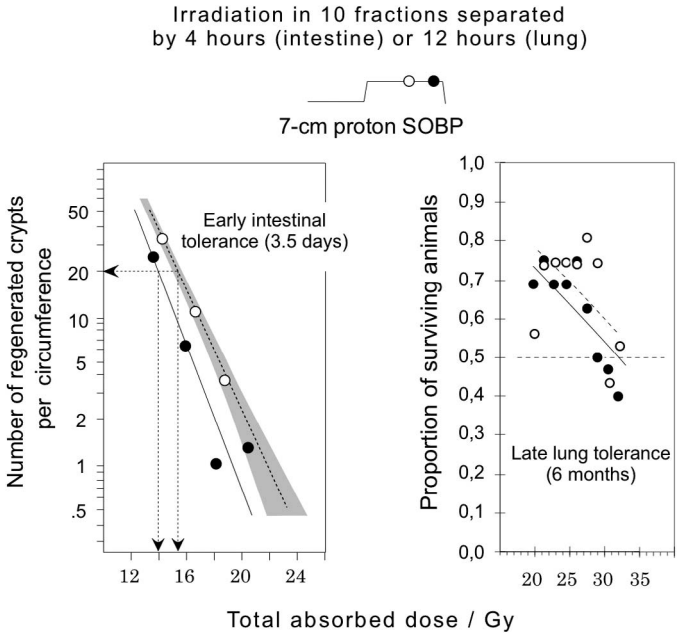

Figure 4. Dose-effect relationships for intestinal crypts regeneration in mice after whole-body irradiations and LD50/180 values for mice after selective irradiation of the thorax. Irradiations were performed in 10 fractions in the 200 MeV energy-modulated proton beam at iThemba LABS. In both cases the animals were positioned in the middle or at the end of a 7-cm SOBP. The propensity for irradiation at the end of the SOBP to be more effective than at the middle is evident for both biological systems. Abbreviation: SOBP, spread-out Bragg peak.

The question is whether or not the predictions based on microdosimetry measurements would translate into measurable and statistically significant RBE variations that are of clinical relevance. Previous experiments were devoted to the study of RBE variations in the SOBP using total body irradiations given in single or fractionated treatments [3–5]. These investigations showed a significant increase in RBE from the beginning to the end of the SOPB. The same is noted in **Figure 4.** Previous studies did not represent the full variation in RBE as the tissue of interest could not be positioned at the very end of the SOBP. Whole-body irradiations allowed readings to be made to an intestine or lung volume with a minimum thickness of about 1.5 cm. As a consequence, the RBE values estimated using whole-body irradiations are averaged over 1.5 cm of tissue. One may thus consider that this RBE value reflects the radiation quality in the middle of the irradiated volume, that is, approximately 7 mm upstream from the very end of the SOBP. As larger variations in RBE values are expected in the last millimeters of an SOBP, it is important to quantify these using functional segments of intact tissue.

The present ex vivo irradiation technique made it possible to narrow the irradiated volume to 2.5 mm (ie, the diameter of the intestine) and to determine RBE values as close as 1.5 mm upstream from the end of the SOBP. The experimental data shown in **Figure 2** were obtained using either a 7-cm or a 3-cm SOBP. Since, as a first approximation, radiation quality at a given distance upstream from the end of an SOBP is independent from the width of the SOBP, the RBE estimates in these experiments can be pooled.

The increase in RBE from the middle to the end of the 7-cm SOBP (ie, over the last 35 mm of the SOBP) was found to be the same as the RBE increase from the middle to the end of the 3-cm SOBP, that is, over the last 15 mm of the SOBP. This suggests that the rate of increase of the proton RBE is higher in the distal region of the SOBP. This is consistent with the fact that the RBE variation from the middle of the 3-cm SOBP to its intermediate position (ie, over 7.5 mm) already exhibits an increase of 8%.

It is important to note that the different positions in the SOBP we have investigated in the present experiments are situated in the flat part of the physical spread-out Bragg curve. In this region LET values range from approximately 2 to 4 keV/μm. Other studies determine RBE variations in the distal fall-off of the beam where the LET increases to values greater than 10 keV/μm and where RBE is considerably higher [20, 21].

Conclusion

The present results are consistent with in vitro data showing an increase of the proton RBE at the end of the SOBP [2, 20, 22–24]. It also confirms observations we made previously using crypt regeneration after whole-body irradiations.

It is shown that the proton RBE for the early tolerance of normal tissues increases by 8% to 10% over the last 15 mm of the SOBP. The corresponding increase of the biologically effective dose in the SOBP exceeds the dose accuracy standard of

3.5% required in radiation therapy [25] and is thus of clinical importance. Therefore, it is advisable to allow for it when evaluating treatment plans.

ADDITIONAL INFORMATION AND DECLARATIONS

Conflicts of Interest: The authors have no conflicts of interest to disclose.
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