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Measurement of the Biological Effective Dose Ratio (BEDR) for a 70 MeV Proton Beam. A Test Experiment in Preparation for the AD-4/ACE Experiment

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In order to demonstrate that the method proposed for the antiproton irradiation study to be performed at the CERN/AD during the 2003 run cycle has sufficient depth resolution and efficiency to address the question of enhanced biological effect of antiprotons compared to protons, a test experiment was performed using a 70 MeV proton beam at TRIUMF. The beam was modulated using a single thin plastic disc with a cutout. This produced a spread in beam energy, which can also be easily obtained at the AD, and resulted in a spread out Bragg peak with a width of approximately 3 mm.

We demonstrated that this width could easily be resolved with our method. We measured cell survival vs. depth for 8 different doses between 1 Gy and 14 Gy in the Bragg peak as measured with a micro-dosimeter. Because absolute dosimetry was possible in the experiment we can extract values for the Relative Biological Effectiveness according to the standard definition of this quantity and this analysis is explained here using the high quality data set obtained in this test experiment. We also compared the results for cell survival vs. dose from this experiment with previous work performed at the Massachusetts General Hospital in Boston.

Because absolute dosimetry in the Bragg peak is not available for antiprotons, we also show an alternative method of analysis in which all data are plotted relative to the dose delivered to the entrance channel (plateau dose), which can be measured. This leads to the definition of the Biological Effective Dose Ratio (BEDR) and does not require absolute dosimetry. It is this quantity and the comparison of BEDR values for proton and antiproton beams which are at the center of our proposed study in AD-4.

We performed a test experiment with protons to demonstrate that the experimental method is sound, has sufficient depth resolution, and has adequate precision. Figure 1a shows the measured relative dose as a function of depth for the 70 MeV proton experiment performed at TRIUMF. A rotating thin plastic disk with a cutout was used to modulate the energy of the incident beam producing a spread out Bragg peak. A total of 15 separate 1 mm slices of the solidified growth medium containing live cells were analyzed for cell survival after irradiation in the proton beam. The sample depths were chosen to measure biological response in the plateau and peak regions of the dose versus depth curve. Separate exposures of sample tubes containing cells were made at proton doses of approximately 1, 2, 3, 4, 5, 6, 10 and 14 Gy in the peak region.

For preparing the biological samples, cells were maintained as monolayers in MEM (minimal essential medium) supplemented with 10% fetal bovine serum, 2.2 g/l sodium bicarbonate and 0.122 mg/ml sodium pyruvate. Appropriate numbers of cells were seeded approximately 48 hours prior to irradiation at cell densities designed to give 50 – 75% coverage of the culture surface at the time of harvest. Cell monolayers were harvested with trypsin, neutralized with growth medium, centrifuged and re-suspended in medium containing 12% gelatin and 20% serum at 37 degrees C at a concentration of approximately 2 x 10^6 cells/ml. At this temperature the gelatin was a viscous fluid that was poured into ABS (acrylonitrile butadiene styrene) plastic tubes of 0.8 cm diameter and 18 cm length with a piston located so as to accommodate a 6 cm deep cylinder of gel/medium. After solidification of the gel at 4 degrees C the tubes were sealed and stored on ice.

For irradiation, each tube of live cells was placed within a circulating water bath phantom, approximately 18cm x 18cm x 24cm. A refrigerated glycerine and water solution of the same density as the gelatin and ABS tubes was used to maintain the cells at 2 degrees C. This ensured that at any given depth, the stopping power was independent of lateral position and thus avoids any artifacts that could result from scattering of protons from points outside the gel. The experimental system was very similar to what was used previously to successfully measure the RBE for heavy charged particles [1,2,3]. The sample tubes were held at 2 degrees Celsius until the cells were plated after irradiation.

After irradiation, and under sterile tissue culture conditions, the gel was extruded from the tube using a plunger connected to a delivery mechanism that advanced the cylinder of gel in 1 mm steps. The gel was sliced every 1 mm using a taut wire. The slices from selected depths were dispersed in MEM culture medium containing 10% fetal bovine serum and antibiotics. Each slice represents a different depth along the path of the beam. The dispersed cell samples were analyzed using a cell sorter (flow cytometer), which identified individual cells on the basis of forward and perpendicular light scattering without the use of a cell stain, and dispensed the prescribed number of cells into a test tube for plating. Variable numbers of starting cell densities, depending on their predicted survival, were used to always produce approximately 400 colonies/10 cm dish. This leads to low and stable statistical errors in the raw data and is an extremely accurate way of determining the number of cells plated per dish [2]. This technique is known to improve precision in measurements of RBE values to plus or minus 2 - 4% when folded into the accuracy of the absolute dosimetry. After incubation for 5 - 6 days in a controlled environment, the colonies that developed were fixed, stained and counted. Only those colonies having more than 50 cells were counted as having been derived from a single surviving cell (smaller colonies are due to cells that successfully negotiated one to five doublings before their reproductive death).

Figure 1b shows the surviving fraction versus depth for various doses in the Bragg peak region from 1 Gy to 14 Gy. The data clearly demonstrate it is possible to distinguish the biological response in the plateau and peak regions for protons. There should be an even larger effect observed for the cells that will be exposed to the antiproton beam.

To determine the physical dose delivered to the individual slices shown in figure 1a we placed a certified micro-dosimeter immediately behind the entrance window to the phantom and measured the absolute dose at this location. The absorbed dose at any depth into the sample was then based on the measured dose profiles of protons at the given incident energy in water. The doses were corrected for the effects of the entrance window and the density of the growth medium. With this we can determine for each individual slice the survival of cells vs. absorbed dose. Starting from this set of raw data we then combine the results from slice 1 through 4 to obtain the survival fraction of cells

vs. dose for the plateau region. The average of slice 8 and 9 is used to determine the same relationship for the peak region. The data were fit with the standard linear quadratic model in dose D where S is the surviving fraction:

$$S = S_0 \exp(\alpha D + \beta D^2).$$

Figure 2 shows the results of this analysis for the 70 MeV demonstration experiment with protons, including the cellular responses to ^{60}Co gamma radiation. Through the use of the cell counter the raw data already have high statistical significance and this averaging process further removes remaining small fluctuations visible in figure 1b, arriving at the high quality data set of figure 2.

Because we have absolute dosimetry available for both peak and plateau measurements we can directly obtain the RBE for protons by taking the ratio of the ^{60}Co dose to the peak and plateau doses for protons resulting in identical cell survival. We cannot quote the final result at this time because during our data analysis we became aware of an calibration error in the micro-dosimeter. We will have to reanalyze all data from the beginning, arriving at a (slightly) different figure 2, from which a correct absolute RBE for protons in the peak and the plateau can be extracted.

For antiprotons no data on dose profile vs. depth in biological medium exist and we can therefore not determine the absolute absorbed dose for the different slices. Furthermore, the requested beam time is not sufficient to measure the dose profile. Instead, we introduce a new quantity, the biological effective dose ratio (BEDR), which will allow us to extract meaningful biological data without absolute dosimetry. Because antiprotons behave nearly identical to protons in the plateau region we can use standard dosimetry methods to determine the relative plateau dose. If we then plot the cell survival in the peak and plateau region versus plateau dose we can extract the BEDR, defined as the ratio of plateau doses producing identical biological effects (figure 3). BEDR is an estimate of the “tumor to normal tissue” biological effectiveness for a given incident beam and can be accurately determined without absolute dosimetry. BEDR is directly related to RBE and the RBE can be determined through determination of the peak dose using Monte Carlo modeling. BEDR is a convolution of the ratio of relative

biological effects per particle for peak to plateau and the ratio of the absorbed dose in peak and plateau. At this time we do not intend to unfold these contributions but will concentrate on the accurate determination of the combined effect described by the BEDR. As shown in figure 4, we establish the 95% confidence limits for the plateau and peak survival curves based on the uncertainty in the LQ model fits and then use these error bands to determine the uncertainty in BEDR.

References:

[1] L. D. Skarsgard, et. al., "Radiobiology of pions at TRIUMF"; Int J Rad Biol. Phys. **8** (12), 2127-2132 (1982).

[2] B. G. Wouters, et. al., "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"; Rad. Res., **146,** 159-170 (1996).

[3] L. D. Skarsgard, "Radiobiology with heavy charged particles: a historical review"; Physica Medica **14**, Suppl. 1, 1-19 (1998).

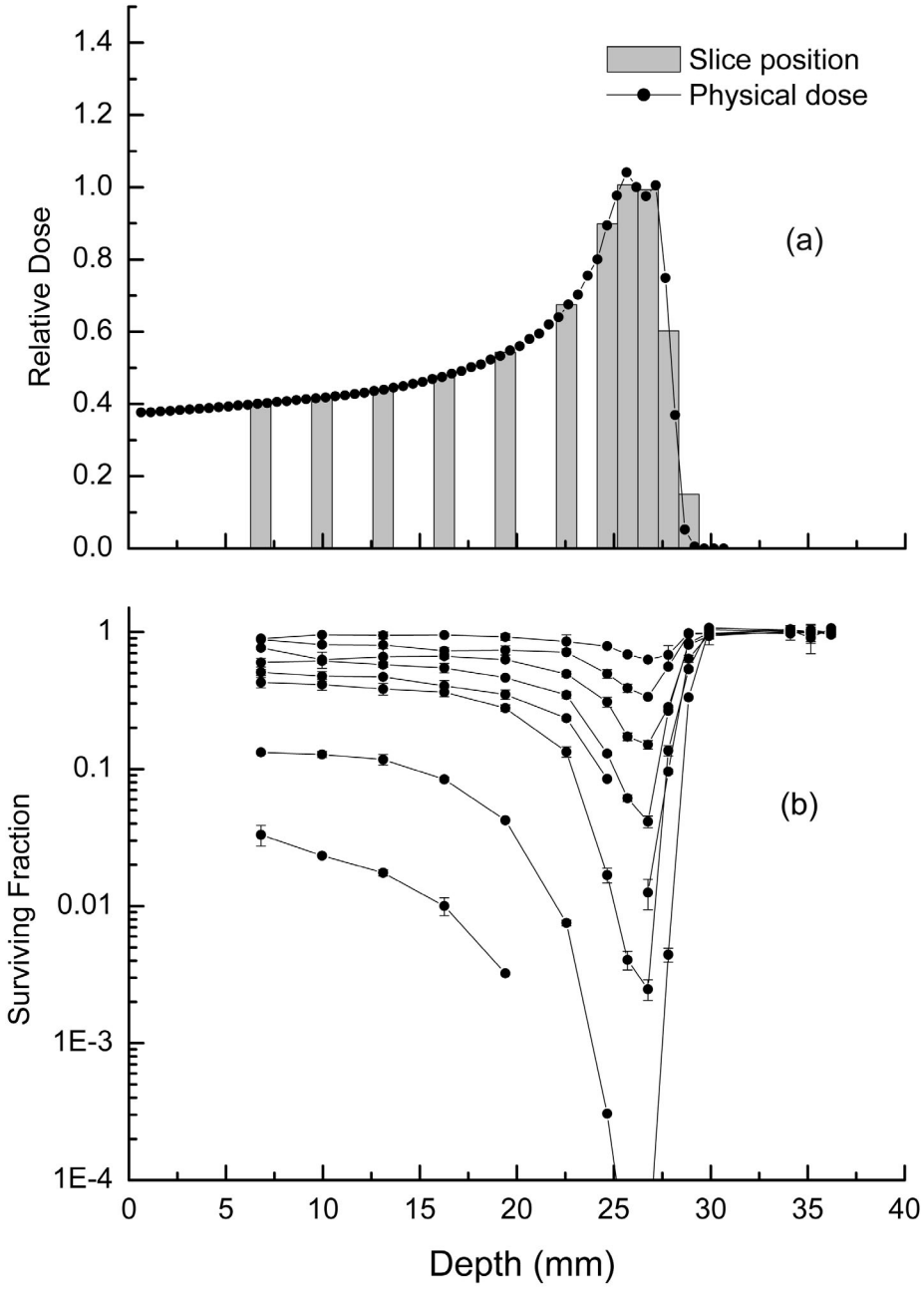

Figure 1. a) Relative dose vs. depth for a two-step spread out Bragg peak (70 MeV proton beam at TRIUMF).

b) Surviving fraction versus depth for doses in the Bragg peak region of approximately 1, 2, 3, 4, 5, 6, 10, and 14 Gy.

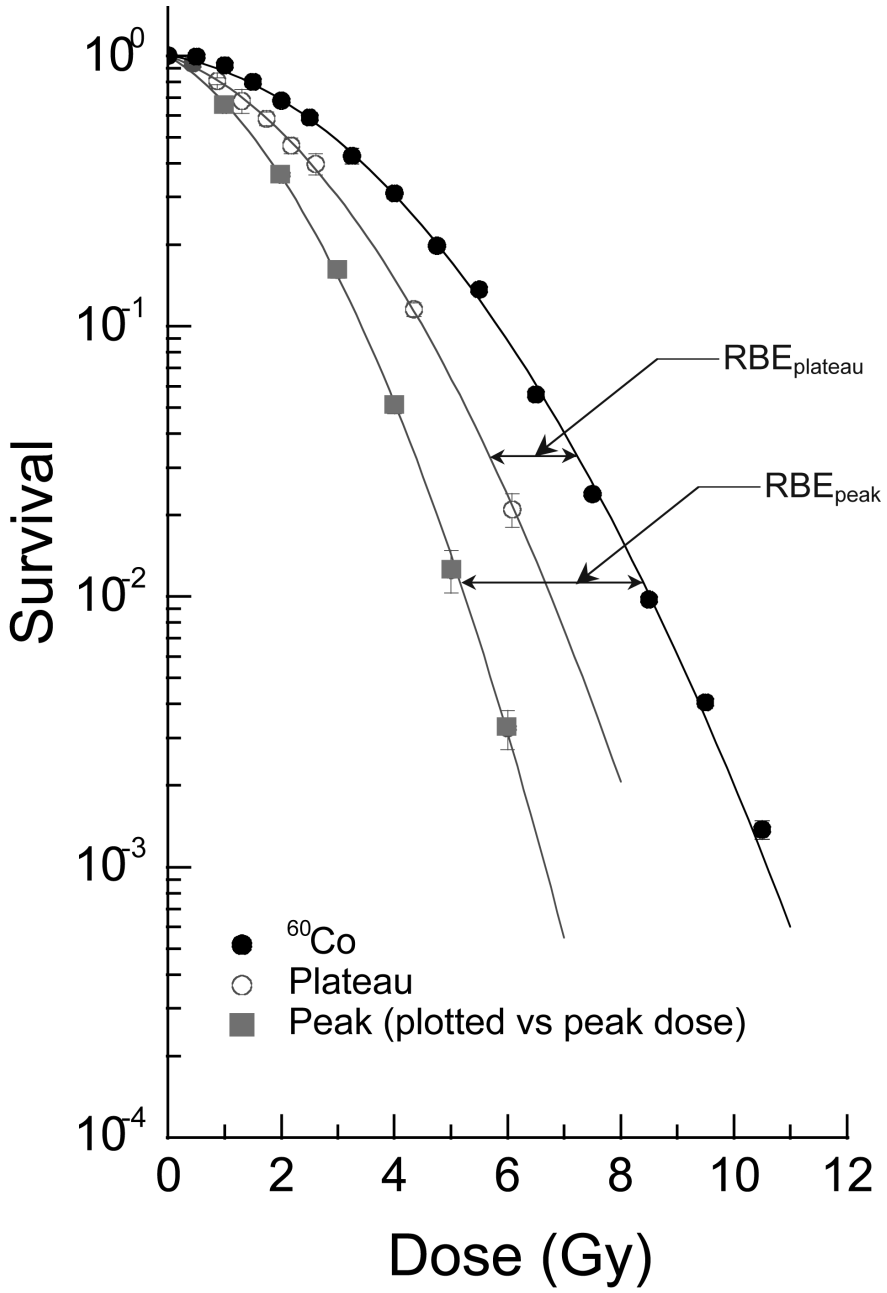

Figure 2. Determination of RBE for the 70 MeV proton experiment.

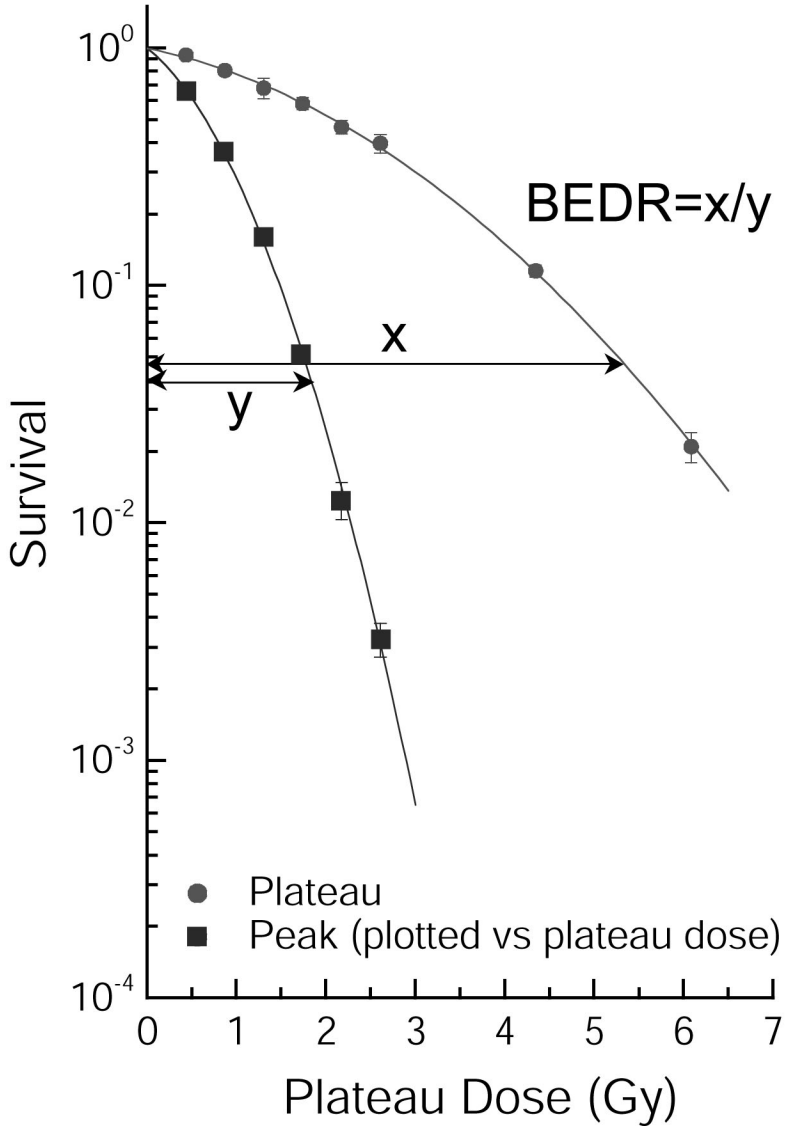

Figure 3. Determination of BEDR (biologically effective dose ratio) without detailed knowledge of the absolute dosimetry within the Bragg peak.

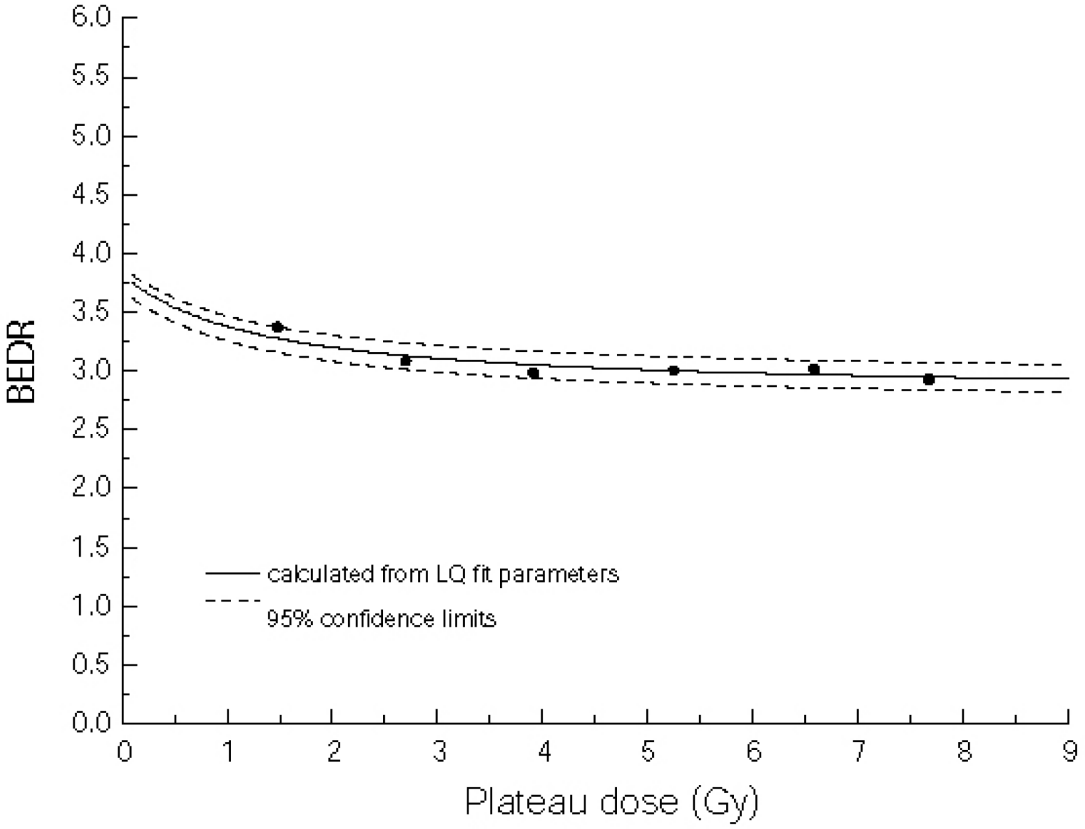

Figure 4. Confidence limits are determined from the uncertainties in the LQ (linear quadratic) model fits to the cell survival curves.