Chapter

Relative Biological Effectiveness Studies Using 3 MeV Proton Beam from Folded Tandem Ion Accelerator: An Experimental and Theoretical Approach

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Abstract

Proton being the easiest light ion to accelerate and achieve desired beam profile, has been pursued as a popular particulate radiation for therapy applications. In the present study, *Saccharomyces cerevisiae* D7 strain was used to estimate the RBE values of the 3 MeV proton beam, and an attempt was made to derive mathematical formula for calculating RBE value with respect to the dose. Dosimetry studies were carried out using Fricke dosimetry and Semiconductor Surface Barrier detector to calibrate the absorbed doses of Gamma chamber-1200 and Folded Tandem Ion Accelerator respectively. Gold standard cell survival assay and gene conversion assay were used to compare gamma and proton radiation induced cell death and genetic endpoint. Multi target single hit model was used to derive mathematical formula for RBE estimation. The results show a linear survival-dose response after proton radiation and sigmoid survival-dose response after gamma radiation treatment. The calculated RBE value from the survival and gene conversion studies was 1.60 and 3.93, respectively. The derived mathematical formula is very useful in calculating RBE value, which varies from 3.61 to 1.80 with increasing dose. The estimated RBE value from the mathematical formula is comparable with the experimental values. With the help of the present mathematical formulation, RBE value at any dose can be calculated in the exponential and sigmoidal regions of the survival curve without actually extending the experiment in that dose region, which is not possible using conventional methods.

Keywords: *Saccharomyces cerevisiae*, relative biological effectiveness, radiation dose, cell survival and gene conversion

1. Introduction

Biological effects of heavy charged particles on humans play an important role in two different scientific fields; in radiation therapy using protons and heavier ions

and in space research for understanding effects on space travelers from galactic cosmic radiation [1]. In addition, the low energy heavy ion accelerators have an important role in basic and applied sciences [2]. Proton being the easiest light ion to accelerate and achieve desired beam profile, has been pursued as a popular particulate radiation for therapeutic applications. Nonetheless, very less has been understood about biological effectiveness of these charged particles. Proton beams can provide highly localized, uniform doses of radiation to tumors, while sparing the surrounding normal tissues, compared with conventional modalities using photons or electrons [3]. In addition to therapeutic applications, energetic proton also finds its presence in space research, neutron dosimetry wherein due to elastic scattering of energetic neutrons lead to (n, p) reaction and creation of low energy protons in the tissues.

The radiobiological studies conforms that equal physical doses of different types of radiation do not produce equal biological effects, because of differences in their energy deposition patterns. This is taken into account by the concept of Relative Biological Effectiveness (RBE). RBE compares the severity of damage induced by a radiation under test, at a dose D_T relative to the reference radiation dose D_R for producing same biological effect. The reference radiation is commonly ^{60}Co-gamma radiation. Generally, the RBE depends on many factors such as the radiation dose, linear energy transfer (LET) at a given tissue depth, dose rate, energy of the radiation, test system and studied biological endpoint. The RBE values of the radiation are very useful in risk estimation during accidental exposure of ionizing radiation (IR) [4]. Revisions in weighting factors for intermediate and very high energy neutrons as well as accelerated protons in the recent ICRP recommendation has drawn more attention to mechanistic approach of studies using radiobiological endpoints.

In the present study, *Saccharomyces cerevisiae* D7 strain was used to study biological effects of 3 MeV proton radiation using cell survival and gene conversion endpoints. The results obtained were compared with standard ^{60}Co gamma radiation. An attempt has been made to estimate RBE value for 3 MeV proton radiation and variation of RBE value as a function of dose with experimental and theoretical formulations. The model organism considered in the study is Saccharomcyces cerevisiae (budding yeast), which is a useful model for higher eukaryotic organisms study and plays a vital role in modern day research. The conservation of many processes such as replication, DNA damage, replication checkpoints and cell cycle control is observed in Saccharomcyces cerevisiae [5]. Additionally, it has been shown that 42% of yeast genes that cause chromosome instability are conserved in humans, demonstrating the importance of yeast in the study of genomic instability and cancer [6]. The prevalence of budding yeast in research today can also be attributed to the low cost at which, experimental procedures can be completed, coupled with its relatively quick doubling time [7].

2. Materials and methods

Gamma source and Dosimetry: ^{60}Co-gamma chamber-1200 supplied by the Isotope Division, Bhabha Atomic Research Center was used for irradiating the cell samples. Fricke dosimetry system was used to calibrate the gamma chamber; the details were given elsewhere [8–15].

Proton Beam Source and Dosimetry: Proton beams are accelerated using the Folded Tandem Ion Accelerator (FOTIA), a facility at Bhabha Atomic Research Centre (BARC), is an electrostatic accelerator with a maximum terminal voltage of 6 MV [2]. Dosimetry of FOTIA was carried out using 2 Silicon Surface Barrier (SSB)

detectors, one mounted inside scattering chamber (Monitor detector) and other on sample position (Sample detector). Position of the beam was visualized using a quartz crystal mounted on a movable ladder in a general purpose scattering chamber maintained in ultra-high vacuum. The pencil beam was made to channel through blank position on the ladder and passed through drift tube of length of about 3 meters. The primary beam was then made to pass through titanium window at the end of drift tube. The beam position was again visualized by keeping a quartz plate after the window. The position of beam was adjusted to the center of the window using X-Y steerer magnets and focused using a quadrupole magnet. Once the beam was tuned to desired geometry and position, the ladder in the scattering chamber was moved so that beam passed through a gold foil of 500 ng/cm^2 thickness. The diffused beam facilitated uniform beam profile at the titanium window. An SSB detector inside the scattering chamber kept at an angle from gold foil helped to monitor the fluence. Another SSB detector was positioned at the position where samples could be mounted, simulating the geometry of sample. The detector was provided with a calibrated collimator to reduce count rate and the fluence measurement was normalized between the two detectors. The profile of the beam was measured by scanning the entire area of titanium window. Intensity of beam was adjusted by varying ion source current. LET measurements were done using TRIM software. The position of the monitor detector was adjusted in such a way that the count rate and dead time of the detector are acceptable. Initial signals from the detector were amplified and digitalized using Multi Channel Analyzer (MCA). The number of scattered proton particles (Monitor proton counts) and diffused proton particles (Sample proton counts) were counted for 100 sec with multiple trials to get the ratio. The fluence of the proton beam at source detector was calculated to measure absorbed dose.

Absorbed dose was calculated using the relation [Kraft et al. 1989]

$$\text{Dose} = \left[\left(1.6 \times 10^{-8}\right) \times LET\left(eV\mathring{A}^{-1}\right) \times \phi\left(\text{particles cm}^{-2}\right)\right] \tag{1}$$

Where fluence represents particles delivered per unit area and LET represents energy transferred per unit length. The LET of the present setup was estimated to be 13 KeV/μm. The fluence of the source detector was measured using

$$\text{Fluence (F) Source Detector} = \frac{[\text{No.of particles on source detector}]}{(\pi \times r_S{}^2)} \tag{2}$$

Where 'r_s' represents, sample detector collimator radius. Number of particles on sample detector can be calculated by taking ratio between monitor detector and source detector counts

$$\text{ratio} = \frac{N_M}{N_S} \Leftrightarrow N_S = \frac{N_M}{\text{ratio}} \tag{3}$$

Substituting Eqs. (3) and (2) in (1) gives

$$\text{Dose} = \left[\frac{\left(1.6 \times 10^{-8}\right) \times 13 \times N_M}{(\pi \times r_S{}^2 \times \text{ratio})}\right] \tag{4}$$

Rearranging Eq. (4), gives

$$\text{N}_M = \left[\frac{Dose \times \pi \times r_S{}^2 \times \text{ratio}}{\left(1.6 \times 10^{-8}\right) \times 13}\right] \tag{5}$$

Eq. (5) was used to calculate required number of monitor detector counts for desired absorbed dose.

Sample preparation and irradiation: A mutant type diploid yeast strain, *Saccharomyces cerevisiae* D7 was used for the study. The genotype of the strain is

$$\frac{a}{\propto}\frac{ade2-40}{ade2-119}, \frac{trp5-12}{trp5-27}, \frac{ilv1-92}{ilv1-92}$$

The single cell stationary-phase cultures were obtained by growing the cells Yeast extract: Peptone: Dextrose (YEPD) (1%:2%:2%) medium for several generations to a density of approximately 3×10^8 cells mL^{-1}. Cells were washed thrice by centrifugation (2000 g for 5 min) and re-suspended to a cell concentration of 1×10^8 cells mL^{-1} (by counting in heamocytometer) in sterile double distilled water. For proton radiation, the cell suspension was mixed well and exactly 1×10^8 cells were filtered using millipore filter assembly in aseptic condition. The filter paper having cells on the surface was placed inside sterile 3 cm diameter petri dish and irradiated for different doses. For gamma ray irradiation, polypropylene vials were used containing 1×10^8 cells per ml. Cell suspensions were maintained at 0–4° C before and after irradiation till plating.

Survival assay and Gene conversion assay: Treated and untreated samples were suitably diluted and plated in quadruplicate on YEPD agar medium. Plates were incubated for 2–3 days at 30°C in the dark, and the colonies were counted. The gene conversion assay was conducted by plating 1×10^6 cells per plate on Trp^-agar medium and incubated for 72 h at 30°C in the dark, and the colonies were counted.

3. Results and discussion

Calibration of ^{60}Co-1200 Source: In the present study, vials containing Fricke dosimetry solution were exposed to gamma rays for different time interval. Optical absorbance measurements of the dosimeter were done at 304 nm wavelength using a UV–Visible spectrophotometer. The absorbed dose was calculated using optical absorbance and is presented in **Figure 1**. Dose response was considered to be best fit with linear model, with a regression coefficient equal to 0.99. The dose rate of the gamma chamber was determined by the same method and was found to be 51 Gy min^{-1}. The dose calibrations are traceable to the National standards.

Calibration of FOTIA: The beam profile of the FOTIA was measured using a collimated SSB detector. The dosimetry methods followed is presented in materials and methods section, uniformity of the beam at sample position was measured by placing sample detector vertically and horizontally at different positions from the central axis.

Cell Inactivation Studies: The survival response of *Saccharomyces cerevisiae* D7 strain after irradiation with proton and gamma radiation is presented in **Figure 2**. It is clear from **Figure 3** that dose response with proton beam is linear, whereas with gamma radiation it is sigmoid. The sigmoid dose response is due to the multi-track hit processes combined with dose rate dependent molecular repair processes [9–11]. The linear dose–response is due to the lethal damage which leads to cell death even at lower doses. The absence of shoulder indicates absence of sub lethal damage repair in the case of proton radiation, whereas for gamma radiation, the shoulder indicates that most of the induced sublethal damages were easily repaired at lower doses.

The obtained experimental data were fit to multi-target single hit theory and the survival response of gamma and proton radiation were represented as

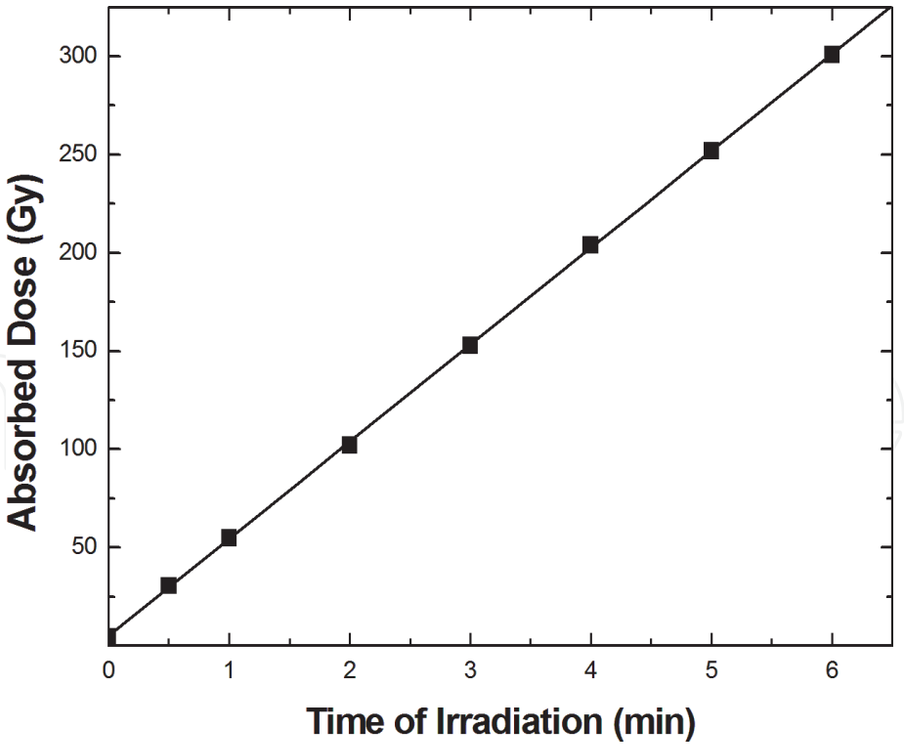

Figure 1.
Dose calibration curve for ^{60}Co gamma Chamber-1200.

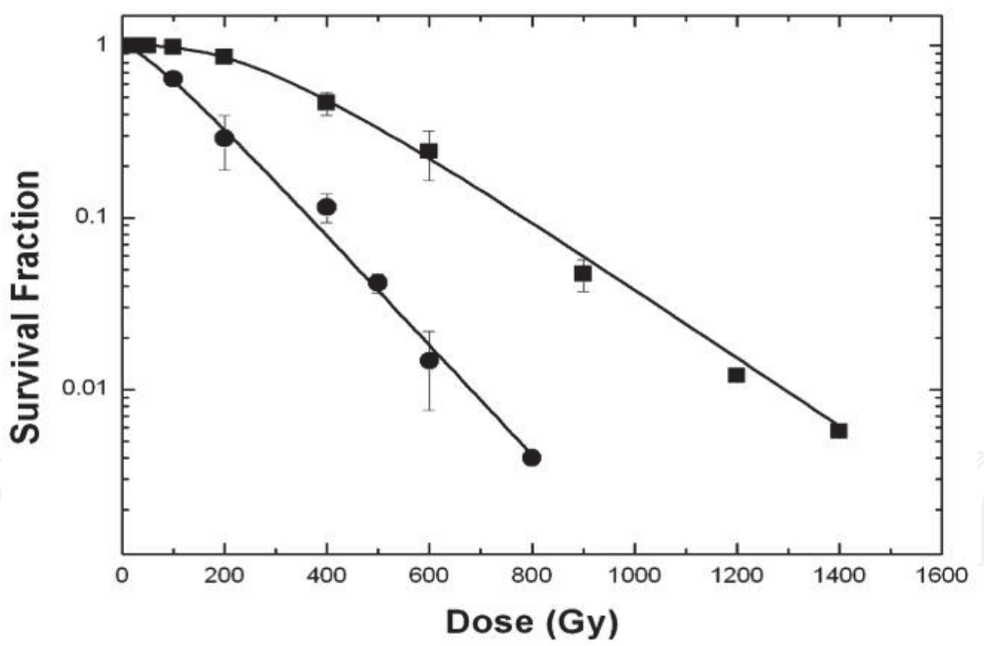

Figure 2.
Dose–response relation after irradiation with proton (●) and gamma radiation (■).

$S_{Gamma} = (1-(1-\exp(0.00459\ D))^{3.78})$ (with $R^2 = 0.99$ and $Chi^2 = 0.00012$) and $S_{Proton} = (1-(1-\exp(0.00736\ D))^{1.50})$ (with $R^2 = 0.99$ and $Chi^2 = 0.00056$). The calculated D_0 value, which is a reciprocal of the inactivation constant, is 218 and 136 Gy for gamma and proton radiation respectively. The RBE value in the exponential region can be calculated by taking ratio between inactivation constant of gamma and proton radiation and is found to be 1.60.

Gene conversation studies: Gene conversion analysis was carried out using *Saccharomyces cerevisiae* D7 yeast cell line at trp locus. Each colony represents a gene

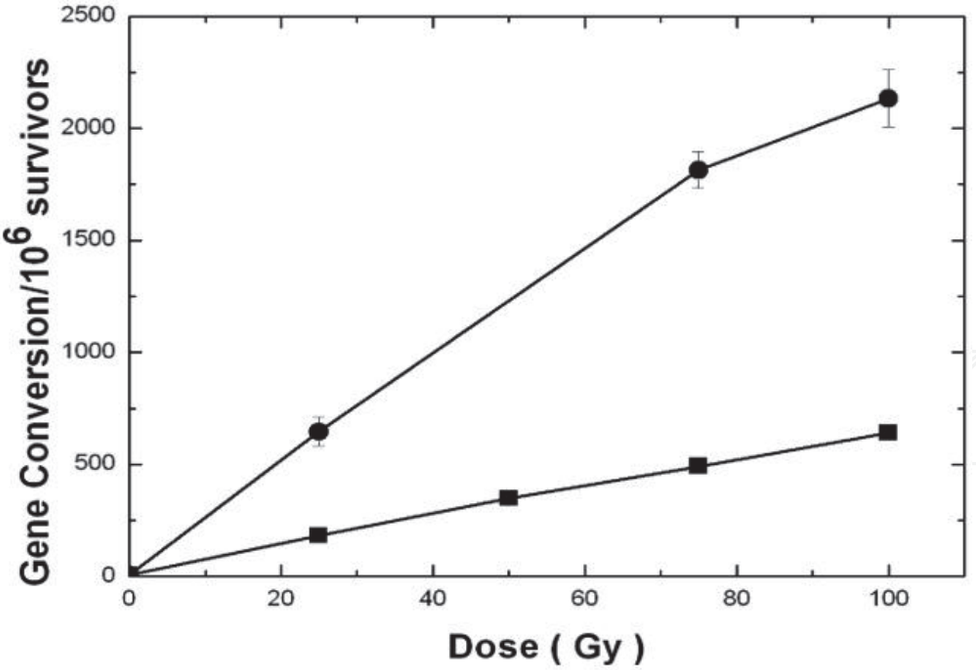

Figure 3.
Gene conversion frequency after irradiating with proton (●) and gamma radiation (■).

convertant and data is presented in **Figure 3**. The doses 25 Gy, 75Gy and 100 Gy were selected and the results show a linear increase in gene conversion frequency with dose. In the case of proton radiation, at lower doses increase in gene conversion frequency was linear, whereas at higher doses it attains plateau. In the case of gamma radiation gene conversion frequency was linear throughout the selected dose region. The gene conversion frequency (G.C.F) for gamma and proton radiation were represented as G.C. $F._{gamma}$ = (6.46 ± 2.19) + (6.46 ± 0.105) D (with R^2 = 0.99) and G.C. $F._{Proton}$ = (7.02 ± 3.44) + (25.44 ± 0.520) D (with R^2 = 0.99). The RBE value of the proton radiation for gene conversion was calculated using slopes, is 3.93.

Relative biological effectiveness studies: In the present study, along with cell inactivation and gene conversion studies, we conducted RBE studies for 3 MeV proton radiations. To estimate RBE value, the experiments were repeated using standard gamma radiation (**Figures 2** and **3**). Estimation of RBE value for proton beam is very important in medical treatment planning, where the RBE values should be known with at least 5–10% accuracy. Generally, a standard RBE value 1.1 is applied to the treatment plan. Recently many authors estimated RBE value for proton beam and they observed that there is a drastic change in RBE value near to Brag's-peak [16–30]. High energy protons have an RBE value of about 1.1, however, for low energy protons still sufficient data is not available to conclude the RBE value. In the present study, we used 3 MeV proton radiation, generally using such energy protons one can observe inside tumor during radiotherapy, so present contributions can be used to strengthen the literature data and can be used to improve proton radiotherapy.

Presently RBE values are calculated on the basis of D_0 doses, which give RBE value in the exponential region. In the present study, we formulated an equation, which can be used to calculate RBE value throughout the selected dose-region. Generally RBE is represented by taking the ratio between gamma radiation and test radiation doses, required to produce the same biological effectiveness.

$$\text{RBE} = \frac{D_G}{D_T} \text{At same biological effectiveness} \qquad (6)$$

Where, D_G is gamma radiation dose and D_T is test radiation (in this case proton radiation) dose. From multi-target single hit model, the survival can be represented as

$$S = \{1 - [1 - \exp(-kD)]^n\} \tag{7}$$

Where S represents survival fraction, k is inactivation constant, D is dose and n gives number of targets. To calculate RBE value, we are considering same survival level with both the radiations, thus using Eq. (7), we can write

$$S_G = S_T$$
$$\{1 - [1 - \exp(-k_G D_G)]^{n_G}\} = \{1 - [1 - \exp(-k_T D_T)]^{n_T}\} \tag{8}$$

Simplifying (8), considering high radiation dose (D)

$$\{1 - n_G \exp(-k_G D_G)\} = \{1 - n_T \exp(-k_T D_T)\}$$
$$\frac{n_G}{n_T} = \left\{\frac{\exp(-k_T D_T)}{\exp(-k_G D_G)}\right\}$$
$$\Rightarrow \frac{n_G}{n_T} = \exp(-k_T D_T + k_G D_G)$$
$$\ln\left(\frac{n_G}{n_T}\right) = (-k_T D_T + k_G D_G)$$
$$\frac{1}{D_T} \times \ln\left(\frac{n_G}{n_T}\right) = \left\{-k_T + \frac{k_G D_G}{D_T}\right\}$$
$$\left\{\left(\frac{1}{D_T}\ln\left(\frac{n_G}{n_T}\right) + k_T\right) = \frac{(k_G \times D_G)}{D_T}\right\}$$
$$RBE = \frac{D_G}{D_T} = \left\{\left[\frac{1}{(D_T \times k_G)} \times \ln\left(\frac{n_G}{n_T}\right)\right] + \frac{k_T}{k_G}\right\} \tag{9}$$

Eq. (9) gives the relation between RBE and dose. In Eq. (9), the D_T, n_T, k_T represents dose, number of target and inactivation constant under test radiation condition respectively and n_G, k_G represents number of target, inactivation constant under gamma radiation condition respectively. The variance in the measurements was calculated using following equations, in Eq. (9) the k_G, n_G, k_T and n_T are variables

$$(\sigma y)^2 = \left\{\left[\left(\frac{\partial y}{\partial k_G}\right)^2 \times (\sigma_{k_G})^2\right] + \left[\left(\frac{\partial y}{\partial k_T}\right)^2 \times (\sigma_{k_T})^2\right] + \left[\left(\frac{\partial y}{\partial n_G}\right)^2 \times (\sigma_{n_G})^2\right] + \left[\left(\frac{\partial y}{\partial n_T}\right)^2 \times (\sigma_{n_T})^2\right]\right\} \tag{10}$$

where y represents RBE value

$$\left(\frac{\partial y}{\partial k_G}\right)^2 = \left\{\left[\frac{-1}{({k_G}^2 \times D_T)} \times \ln\left(\frac{n_G}{n_T}\right)\right] - \frac{k_T}{{k_G}^2}\right\}^2 \tag{11}$$

$$\left(\frac{\partial y}{\partial k_T}\right)^2 = \left(\frac{1}{k_G}\right)^2 \tag{12}$$

$$\left(\frac{\partial y}{\partial n_G}\right)^2 = \left(\frac{1}{n_G \times k_G \times D_T}\right)^2 \tag{13}$$

$$\left(\frac{\partial y}{\partial n_T}\right)^2 = \left(\frac{1}{n_T \times D_T \times k_G}\right)^2 \tag{14}$$

Accordingly standard deviation was calculated. **Figure 4** represents RBE value of 3 MeV proton beam at different doses, calculated using Eq. (9). The experimentally calculated RBE value and theoretically calculated RBE values were compared and presented in **Figure 4**. Very good correlation between experimental and theoretical data was observed.

Higher RBE values were observed at lower doses whereas remain constant at higher doses. RBE values varied in a range from 3.61 to 1.80; the maximum value at lower doses is mainly due to the absence of sub-lethal repair processes. In the case of gamma radiation, at lower doses induced damages are repaired but in the case of proton radiation a small dose also creates lethal damages, hence maximum RBE value was observed. At higher doses the damage due to peroxyl radicals and multi-ionizing events lead to lethal damage in gamma radiation, hence RBE value remains constant. Another reason for higher RBE value is energy deposition pattern of the 3 MeV proton radiation. The LET of the gamma radiation is 0.2–0.3 keV μm^{-1}, whereas 3 MeV proton radiation is 13 keV μm^{-1}. The higher RBE values for low energy protons were reported previously [16–30]. Belli et al. [16] has reported that the RBE depends on LET of the proton radiation. They studied SOBP region proton radiation using V79–753B cells. The RBE value for protons with LET 7.7 keV μm^{-1}, 11 keV μm^{-1}, 20 keV μm^{-1}, 30.5 keV μm^{-1}, 34.6 keV μm^{-1} and 37.8 keV μm^{-1} is 2.22 ± 0.27, 2.88 ± 0.37, 3.64 ± 0.41, 5.59 ± 0.54, 5.06 ± 0.51 and 4.50 ± 0.44, respectively [16]. Similar type observation was made by Folkard et al. [17] and reported an RBE value for protons with mean energies of 1.9, 1.15 and 0.76 MeV, using V79 chinese hamster cells. The RBE values for cell survival at 10% survival level are 1.6, 1.9 and 3.36 for protons with track-average LETs of 17, 24 and 32 keV μm^{-1}, respectively.

In another report Mark Andrew [28] observed an RBE value of 2.6 ± 0.6 for 94 keV, 3.1 ± 0.4 for 250 keV, 3.9 ± 0.8 for 390 keV and 2.4 ± 0.5 for 1.2 MeV protons using V79 cell line. Belli et al. [18] studied four human cell lines, SCC25, SQ20B

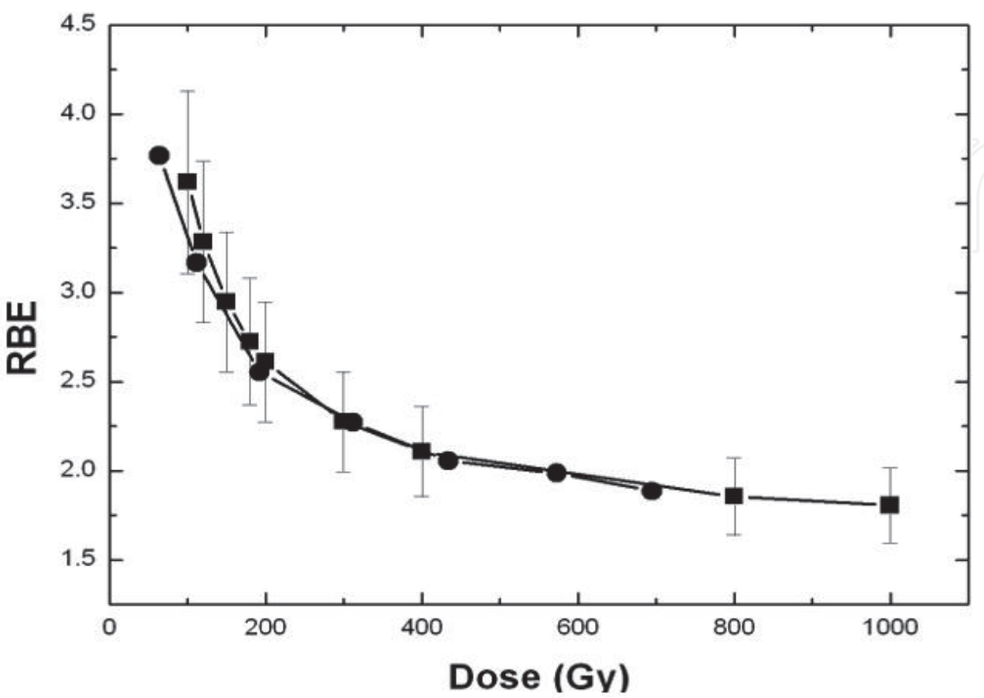

Figure 4.
Variation of RBE with dose; experimental (●) and theoretical (■).

derived from human epithelium tumors of the tongue and larynx, respectively, the normal lines M/10, derived from human mammary epithelium, and HF19 derived from a lung fibroblast. The RBE of the proton beams with LET 30 keV μm^{-1} was 3.2, 1.8, 1.3 and 0.8 for SQ20B, M/10, SCC25, and HF19, respectively [18]. Similarly, Ristić-Fira et al. [29] reported RBE value for mid SOBP region proton particles using radio-resistant human HTB140 melanoma cells and is found to be 2.09 ± 0.36.

Recently, Wéra et al. [30] irradiated Human A549 alveolar adenocarcinoma cells with 4 MeV broad proton beam and calculated RBE value at 10% survival. They reported RBE value of the low energy proton radiation is independent of the dose rate and is equal to 1.9 ± 0.4 for 10 keV μm^{-1} and 2.9 ± 0.5 for 25 keV μm^{-1} [30]. In the same study they calculated RBE values at 77% survival level and were equal to 10.7 ± 3.3 and 3.6 ± 0.6 for 10 keV μm^{-1} and 25 keV μm^{-1} respectively [30]. These values suggest that RBE value depends on survival, which again depends on radiation dose. Britten et al. [22] studied human Hep2 laryngeal cancer cells and V79 cells at various positions along the SOBPs of beams with incident energies of 87 and 200 MeV. Using Hep2 cells, the RBE values were 1.46 at the middle of SOBP, 2.3 at the distal end of the SOBP [22]. For V79 cells, the RBE for the 87 MeV beams was 1.23 for the proximal end of the SOBP, 1.46 for the distal SOBP and 1.78 for the distal end of the SOBP [22]. Similar studies were conducted by Paganetti [23], Słonina et al. [24] and Aoki-Nakano et al. [26] to calculate SOBP region RBE value. They concluded that, the proton RBE value increases with increasing LET which ranges from 1.1 to 4.98. The RBE values for continuous and pulsed proton radiation also studied using human tumor cells [27]. No significant difference was observed between pulsed proton (RBE = 1.22 ± 0.19) and continuous proton (RBE = 1.10 ± 0.1) beam [27].

Previous studies reveal that there is a large variation in reported RBE values among laboratories with the same cell line and a similar LET. For example, Belli et al. [16] and Folkard et al. [17] measured an RBE value of 24 keV μm^{-1} protons as 1.9 and 2.4, respectively. On average, literature reported data concludes RBE value for low energy proton radiation varies from 0.9 to 6, which is comparable with the present findings.

4. Conclusion

The study confirms that, the 3 MeV proton beam is more lethal to biological system compare to gamma radiation and the dose response was found to be linear. Nearly 4 times higher gene conversion frequency was observed in proton radiation as compared to gamma radiation. The estimated RBE value estimated from the mathematical equation developed in the present study is comparable with the experimental values. The RBE value of the 3 MeV protons was found to decreases with the dose and varied from 3.61 to 1.80. With the help of the present mathematical formulation, RBE value at any dose can be calculated in the exponential region of the survival curve without actually extending the experiment in that dose region, which is not possible using conventional methods.

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