Iranian Journal of Medical Physics

ijmp.mums.ac.ir

Assessment of in Vitro Radiosensitivity Parameters of Breast Cancer Cells Following Exposure to Radiotherapy Hospital-Based Facilities

Samaneh Babazadeh Toloti[1], Laleh Rafat Motavalli[1], Hashem Miri Hakimabad[1], Samira Mohammadi Yeganeh[2,3], Eftekhar Rajab Bolokat[4]

1. Physics Department, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Cellular and Molecular Biology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Radiation Oncology, Shohada-e-Tajrish Hospital, Tehran, Iran

ARTICLE INFO

Article type:
Original Article

Article history:
Received: Nov 13, 2017
Accepted: Jan 26, 2018

Keywords:
Radiotherapy
Relative Biological Effectiveness
Cell Survival
In Vitro Techniques

ABSTRACT

Introduction: The aim of the present study was to assess the radiosensitivity parameters for SK-BR-3 (SKBR3) breast cancer cells that could be implemented in the cutting-edge treatment planning systems (TPS) for accelerated partial-breast irradiation (APBI).

Materials and Methods: The cell survival fraction and its relevant radiosensitivity coefficients, namely α and β, in linear-quadratic (LQ) formalism were evaluated for 6 MV X-rays and ^{60}Co γ-rays using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. During the irradiation time, the medium temperature was kept at 4°C to prevent the repair of sublethal radiation damages over the exposure time and keep the survival fractions independent of the dose rate.

Results: Fitting the LQ model to experimental data, α, β, and α/β radiosensitivity parameters were obtained as 0.156±0.027 Gy^{-1}, 0.026±0.007 Gy^{-2}, and 6.0 Gy for 6 MV X-rays and 0.162±0.028 Gy^{-1}, 0.028±0.007 Gy^{-2}, and 5.8 Gy for ^{60}Co gamma radiation, respectively. The average relative biological effectiveness (RBE) values were 0.91 and 0.96 for 6 MV X-rays and ^{60}Co γ-rays, respectively. The derived LQ parameters were also compared with those previously obtained from in vitro studies for different breast cancer cell lines using various regimes, such as radiotherapy modality with different dose rates and delivered doses.

Conclusion: The results of this study provided essential constant values for α and β parameters. The data could be useful for the improvement of TPS to include the effect of different biological responses to radiation in APBI treatment plans.

► *Please cite this article as:*
Babazadeh Toloti S, Rafat Motavalli L, Miri Hakimabad H, Mohammadi YeganehS, Rajab Bolokat E. Assessment of In Vitro Radiosensitivity Parameters of Breast Cancer Cells Following Exposure to Radiotherapy Hospital-Based Facilities. Iran J Med Phys 2018; 15: 132-139. 10.22038/ijmp.2018.26394.1267.

Introduction

The application of accelerated partial breast irradiation (APBI) in the treatment of breast cancer is an issue of increasing interest [1-3]. The radiobiological effect of radiation on the target cells was simply taken into account in the high dose rate (HDR) brachytherapy (BT) treatment planning systems (TPS). The treatment plans are conventionally optimized with respect to dose homogeneity, while the biologic effects of various dose rates are ignored. The improvement of the TPS to a level in which optimization is accomplished according to isoeffect dose surfaces requires the provision of the linear-quadratic (LQ)-associated radiosensitivity parameters, namely α, β, and α/β.

Several studies have assessed the radiosensitivity to the photon irradiation of breast cancer cells. In this regard, Ruiz de Almodóvar et al. [4] reported the α value range of 0.1-0.6 Gy^{-1} for five different breast cancer cell lines treated by a Cobalt-60 (^{60}Co) source at the HDR of 7 Gy/min. Marthinsen et al. [5] investigated radiation impacts on two different breast cancer cell lines after photon irradiation of either 6 MV linear accelerator (with either HDR of 6 Gy/min or low dose rate of 0.2 Gy/min), 380 keV (average dose rate of 0.2 Gy/min) from a ^{192}Ir BT source or 50 kV miniature isotropic X-ray source at an average dose rate of 0.2 Gy/min. Their obtained α and α/β values ranged within 0.12-0.74 Gy^{-1} and 2.2-96 Gy, respectively. The value obtained for α/β ratio in other in vitro studies conducted on human breast carcinoma cell lines is reported as ~ 4 Gy [4, 6-9].

All these studies were in line with the literature reporting about the variation of the radiation

*Corresponding Author: Physics Department, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran. Tel: +985138796983; Email: rafat@ferdowsi.um.ac.ir

sensitivity parameters of low linear energy transfer (LET) (X- or γ-rays radiation) with dose rate. For a given absorbed γ-ray dose, the level of cell survival decreases as the dose rate increases. This effect is due to the repair of the sublethal radiation damage to DNA over time.

Therefore, α and α/β values show high sensitivity to dose rate, unless the dose reduction factor (DRF, see section 2.7) is explicitly introduced, or the sublethal radiation damage is prevented.

In the first approach, several examinations with different dose rates should be performed to derive DRF values as well. However, in the second one, the experiments are performed under the conditions, which stop the sublethal radiation damages, and the number of examination remains unchanged.

The present study aimed to provide radiosensitivity parameters via investigating the in vitro survival curves of the SK-BR-3 (SKBR3) cancer cells in situations preventing the DNA sublethal repair. In addition, the effect of different single radiation doses of ^{60}Co gamma and 6 MV X-rays was studied. The Monte Carlo simulation of the experiment, including the irradiation facilities, as well as the geometry of the cell lines, was also performed to accurately determine the absorbed dose delivered to the cells.

After securing the dose delivered to the cells, the cellular response of SKBR3 breast cancer cells was determined in terms of cell survival fraction using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay method by exposing the cells to single fraction doses of gamma radiation from a ^{60}Co unit and a 6 MV X-rays linear accelerator.

The obtained radiobiological parameters were compared with those previously reported in the literature. Finally, to compare the effect of the different radiation devices, the RBE values were calculated based on the obtained dose response results. The LQ radiosensitivity parameters, namely α and β, were used for the analysis of RBE.

Materials and Methods

Cell line and cell culture

The human breast cancer cell line, SKBR3, (purchased from National Cell Bank of Iran, Pasteur Institute of Iran, Tehran, Iran) was used. The cells cultured in Dulbecco's modified Eagle's medium were supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (100 µg/mL) in T-25 tissue culture flasks at 5% CO_2 and 95% air in a humidified 37°C incubator. The medium was changed every 2-3 days.

Measurement of SKBR3 cell thickness

The measurement of cell thickness was performed by Confocal Laser Scanning Microscope (CLSM- a Leica TCs SP5II, Wetzlar, Germany) in Core facility, Pasteur Institute of Iran. To this end, the cells with the density of about 8×10^3 cells/cm^2 were plated on glass substrates for 24 h. During the measurement, the cell population growth was in exponential phase. Subsequently, 1 µM fluorescence dye acridine orange (which could be excited by the 488 nm argon laser) was added to the culture medium containing cells. After 10-min incubation at 37°C, the cells were washed with phosphate-buffered saline (PBS) and measured by CLSM.

Confocal microscopy through focusing (CMTF) has been regarded as one of the major advances in confocal imaging techniques. Since the entire points in the CMTF curve have direct correlation with high-resolution images, the precise calculation of the inter-layer distances of the cells can be achieved by utilizing the exact Z-axis position of the cell structure. The interactive viewing of the image associated with the cursor on the Z-curve and evaluation of the distance between any two points on the curve can be realized via specialized software. Due to the difference in the intensity of light reflected from different cell layers, the depth-intensity profile can be employed for determining the cell sublayer location [10].

The Z-stack images, were captured through the entire cell line using an inter-slice distance of 0.3 µm. The maximum height of monolayaer cell line was considered as the cell thickness and calculated by multiplying the number of slices involved by the slice thickness. The uncertainty of the cell thickness measurement was assumed to be identical to the inter-slice distance (i.e. 0.3 µm).

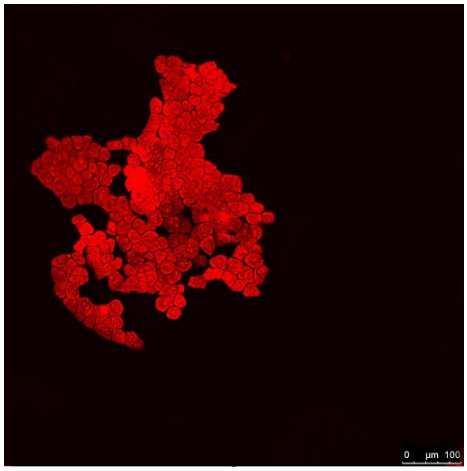

Figure 1. Confocal images of SKBR3 cell line taken in 0.3 µm steps along the Z-axis

Cell growth curve

Growth curve was determined to ensure that the cells were within the exponential growth phase at the irradiation start time. The assessment of cell proliferation was carried out by monitoring MTT conversion into formazan.

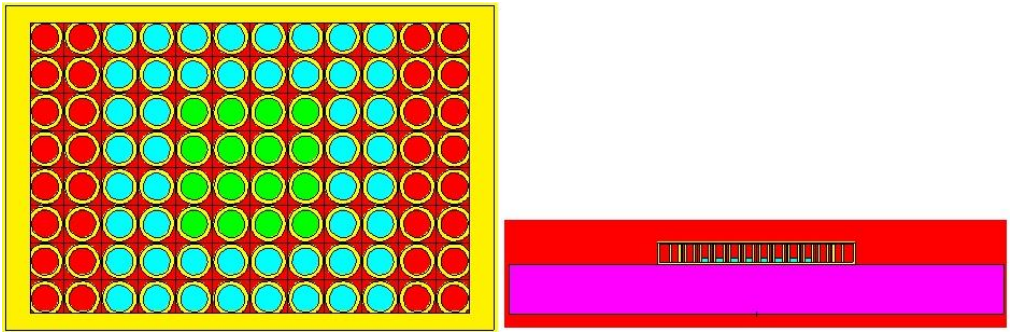

Figure 2. Starting from the left: xy and xz view of the 96-well plate geometry of MCNPX at the irradiation position; air is represented in red; the polystyrene plate is yellow; the Perspex is purple; the culture medium is blue; the cells are green. To view the too thin cell layers in the plot, pz is set at small value, and therefore the culture medium in 16 middle wells cannot be seen in this representation.

The dark-colored formazan dye, which is the basis of MTT assay, was formed as a result of tetrazolium salt MTT reduction by the metabolically active cells.

After 2-3 h of incubation, the water-insoluble formazan dye produced organic solvent-dissolvable crystals, the amount of which could be semi-automatically determined by means of a microplate reader. Since absorbance is associated with the number of the cells, the MTT assay can be applied to evaluate cell growth after irradiation [11]. To this aim, the cells were plated at a density of 5×10^3 cells per well in the 16 middle wells of a 96-well plate to determine the proliferation parameters by the MTT method (as described previously). It was constructed by plotting absorbance (blanked with Dimethyl sulfoxide [DMSO]) against time.

Monte Carlo simulation

MCNPX 2.6.0 is the simulation code employed for the accurate estimation of the radiation dose delivered to the cells. The irradiation facilities (T780 Co-60 unit and a 6 MV photon beam) were modeled in two MCNP input files with the geometry of the setup for cell irradiation. The 96-well plate geometry was simulated as a 0.1-cm thick box (inner size: 11.8×7.8×1 cm) made of polystyrene with the density of 1.04 g/cm^3. The box was filled with non-overlapping cylindrical meshes with the outer and inner diameters of 0.82 and 0.62 cm, respectively (Figure 2).

For the 16 middle wells of the plate, the studied volume contained a culture medium of 0.29 cm thickness in addition to the cells of 7 μm thick monolayer. The two rows around these wells had the same culture medium without cells. The material chosen for the simulated cells and culture medium was provided based on their analysis with Vario EL III Element Analyzer (Table 1).

The simulation was performed in both irradiation setups without Perspex sheet and in the presence of 30×30×3 cm Perspex sheet at the bottom of the plate. The card mode 'pe' was employed in order to transport photons and the secondary electrons responsible for dose delivery in the cells. The energy deposition function (*F8) was used to obtain the doses in unit of MeV per starting particle, which were then converted into gray by considering the information of commissioning data and dose rate of 6 MV X-rays machine or ^{60}Co unit.

Table 1. Materials used for the simulation geometries

Material	Density (g/cm3)	Element	Mass (%)
SKBR3 cell	1.02	H	6.749
		C	43.743
		O	37.445
		N	11.338
		S	0.725
Medium	1	H	11.35
		C	0.494
		O	87.9
		N	0.145
		S	0.111

Cell irradiation setup

Following harvesting with trypsin, the cells were counted and plated in 96-well culture plates with the approximate concentration of 5×10^3 cells/well; then, they were incubated overnight to adhere. On the next day, an hour prior to irradiation, the medium was substituted with 100 μL of 2% FBS-containing medium. The exponentially growing cells were transported from our laboratory to Shohada-e-Tajrish Hospital, Tehran, Iran, on dry ice.

The cells were irradiated by photon beams either from a 6 MV linear accelerator (Elekta Compact) or ^{60}Co unit (Theratron 780C). The 6 MV accelerator operated at the dose rate of 1 Gy/min and source surface distance (SSD) of 100 cm, and the ^{60}Co unit was applied at the dose rate of 0.7 Gy/min and SSD of 80 cm as reference radiation. The ionizing radiation was delivered to the experimental groups as a single dose ranging from 1 to 6 Gy on a 10×10 cm field size. During the irradiation, the plates were vertically situated at the field center with the gantry angle of 180°. This was performed by aligning the marks on the plate with the treatment room lasers.

Perspex sheet (30 mm thick) was used to maintain the conditions of near-equilibrium of secondary charged particles so that the beam was homogenized and flat. To guarantee no photon absorption or scattering by the plate layers, dosimetry was carried

out before cell irradiation using a Farmer chamber (PTW 30013, Germany under Technical Reports Series No. 398 protocol) [12]. Accordingly, the dosimetry results indicated no radiation absorption prior to or following the plate placement.

In order to measure the damages based on factors other than radiation, one plate was not exposed as the control group for each radiation source. All culture plates (including the control non-irradiated plates) were kept at 4°C during the radiation procedure.

Following irradiation, the cells were transported back to the laboratory on dry ice. Then, 100 µl medium containing 18% FBS was immediately added to each well. Finally, they were incubated at 37°C for 7 days due to the creation of a dose-dependent mitotic delay in cells following exposure to various radiation doses. The transportation and irradiation procedure took about 1.5-2 h.

MTT assay and radiation survival fraction

The X- and gamma-ray-induced cytotoxicity was also evaluated by MTT colorimetric assay. To this end, the culture medium in each well was substituted with 100 µl of fetal calf serum-free culture medium and 10 µl of MTT (5 mg/ml in PBS), and then the plates were placed in the incubator again. After 4 h, the medium was removed, and the cells were lysed by 200 µl of DMSO.

Then, formazan product was dissolved, and the absorbance was measured at 570 nm by means of an ELISA reader (Stat Fax-2100 Awareness, Mountain View, CA, USA). Relative survival was defined as the ratio of treated sample to the control group absorbance. Fifteen replicates were employed for each dose group. By the enhancement of cytotoxicity due to radiation exposure, the test group showed a reduction in the level of injected MTT (yellow color) to formazan dyes (purple color), in comparison to the control group. The decrease in metabolic activity appears as the continuous function of deposited radiation energy in the critical target and can be considered as a measure of cytotoxicity [13].

In MTT assay, for each dose level tested, about 5×10^3 cells per 16 middle wells of a 96-well plate were put in 200 µl culture medium. The two rows around these wells were filled with 200 µl culture medium to maintain the humidity and uniformity of the plate during the irradiation treatment under the ^{60}Co γ-ray and 6 MV X-ray sources. The 16 remaining wells kept free of the cells to obtain the reference optical density (OD) from their readings. A cytotoxic event induced by radiation exposure was assumed only when MTT assay recordings were below the_minimum for controls. Survival fraction was evaluated on the 7th day post-radiation exposure. Eventually, surviving fractions were determined by the following formula:

$$\text{Survival fraction} = \frac{\text{mean OD in test wells} - \text{mean OD in cell free wells}}{\text{mean OD in control wells} - \text{mean OD in cell free wells}} \quad (1)$$

Data Analysis

The LQ formulation is often used to model the biological response to radiation [14]. The basic equation of the incomplete repair model for continuous irradiation is:

$$E = e^{(-\alpha D_Z - G\beta D_Z^2)} \quad (2)$$

where E is the level of effect (for instance, surviving fraction), D_Z is the dose of specific radiation Z in Gy, α and β are the parameters of the LQ equation, and G is the DRF describing sublethal damage repair during continuous exposure.

The value of G is defined as the function of recovery halftime ($T_{1/2}$) and the duration of continuous exposure (t) as below:

$$G = 2\frac{[\mu t - 1 + \exp(-\mu t)]}{(\mu t)^2} \quad (3)$$

where $\mu=0.693/T_{1/2}$ [15,16].

During the irradiation procedure, the temperature of the culture medium was maintained at 4°C to prevent any sublethal irradiation damage repair over various exposure times. Therefore, there was no need to correct the variable dose rates of different beams (DRF of 1.0).

To compare the two specific radiations and obtain the biological effect of the test and reference radiations, D_t and D_r should be determined [17]. The RBE is the ratio of the reference radiation dose to the test radiation dose given equal biological effects (isoeffect RBE).

$$RBE = \left[\frac{D_r}{D_t}\right]_{isoeffect} \quad (4)$$

$$= \left[\frac{SF^*}{\alpha_r + \beta_r D_r}\right]\left[\frac{SF^*}{\alpha_t + \beta_t D_t}\right]^{-1} = [\alpha_t + \beta_t D_t][\alpha_r + \beta_r D_r]^{-1} \quad (5)$$

where SF^* is the negative of the natural log of the survival fraction.

Statistical analysis was performed using the SPSS software (version 22) based on the mean and standard deviation of the robustness of the samples. Furthermore, the assessment of the specific effect of the different irradiation protocols was performed in the Origin 8 software.

Comet assay and evaluation of DNA damage

Exposure-induced DNA damages for 2 Gy of ^{60}Co gamma rays were assessed via alkaline Comet assay. After exposure to radiation, the samples were centrifuged at 0°C. The supernatant was removed, and the pelleted cells were mixed with 100 µl of low melting point agarose (0.75% agarose in PBS) at 37°C. Then, the cell mixture was placed on 1% normal agarose-precoated frosted slides (Merck, Germany) and immediately capped with a coverslip.

For the purpose of solidification, the slides were inserted on a tray, which was placed on ice for 15 min. The coverslip was then removed. Afterwards, the slides were submersed in the alkaline lysis solution (2.5 M NaCl, 100 mM ethylenediaminetetraacetic acid [EDTA], 10 mM Tris base, 10% DMSO, 1% Triton X-100, Merck, Germany, pH=10) for 1 h at 4°C. Following lysis process, unwinding step was performed in which the slides were immersed in a fresh alkaline solution (0.3 M NaOH and 1 Mm EDTA; Merck, Germany, pH>13) in a horizontal gel electrophoresis tank (BioRad, USA) for 40 min at 4°C in darkness.

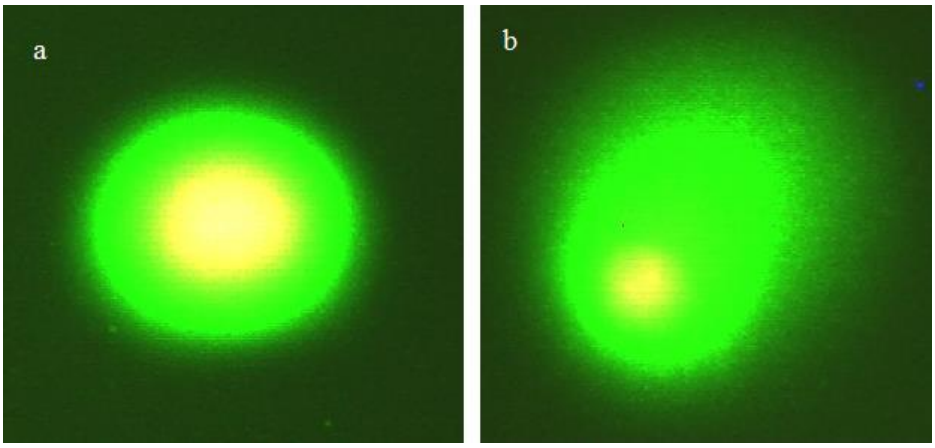

Figure 3. Comets from SKBR3 cells as seen in a fluorescent microscope after lysis and electrophoresis; a- control, b-after exposure to ^{60}Co gamma rays

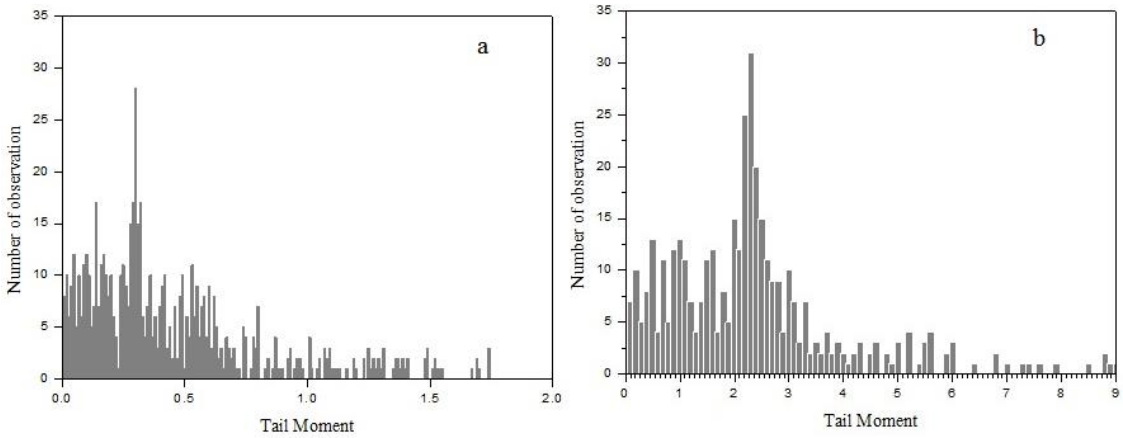

Figure 4. Distribution of DNA damage measured by the alkaline Comet assay in SKBR3 cells: a-control, b- after exposure to ^{60}Co gamma rays. Mean tail moment±SE values of about 100 cells/slide of three independent examinations were approximately 0.3±0.07 and 2.3±0.09 for control group and the group treated with 2 Gy of ^{60}Co, respectively.

Electrophoresis was performed at 0.7-1 V/cm and current of 300 mA for 30 min at the temperature of 4°C. After washing the slides with neutralization buffer (0.4 M Tris buffer, pH=7.5) for three times at room temperature and rinsing them with 70% ethanol for 5 min, they were dried by air.

Cell staining was performed with 20 µl SYBR green (Merck, 2 µg/ml) under a coverslip. For image analysis, the slides were placed under a fluorescent microscope (Nikon, Japan) having a SYBR green filter (excitation filter of 492 nm and emission filter of 521 nm) and a charge-coupled device camera (Figure 3). Several other authors employed this method for assessing the DNA damage through Comet assay [18-20].

The extent of DNA damage could be often evaluated visually through tail moment measurements; however, image analysis software is another available method for the assessment of different parameters. In the present study, DNA strand break formation was assessed as a tail moment evaluated by the Comet assay analysis software. The mean tail moment of about 100 randomly selected cells/slide of three independent examinations were measured for control group and treated with 2 Gy of ^{60}Co (Figure 4). Statistical analysis was performed using the Origin 8 software.

Results

Doubling time

To ensure that the cells were in the exponential growth phase at the irradiation start time, the doubling time of cell line was obtained from the growth curve of the cells. The growth curve for the

SKBR3 cells according to MTT assay after 24, 48, and 72 h of culturing is displayed in Figure 5. Based on this curve, the cell doubling time was 24.6 h.

Calculated delivered dose

The absorbed doses delivered to the cells from both irradiation facilities were obtained by modeling the geometries of the two setups for cell irradiation. The results of these simulations, which were normalized using the commissioning data and dose rates of 6 MV X-ray machine or ^{60}Co unit, are tabulated in Table 2. The associated errors of the outputs were less than 3% in the volumes containing the cells (2.07×10^{-4} cm^3) and less than 0.3% in other studied volumes. Simulation results confirmed that the cell doses were consistent with the experimental delivered doses within 7-8%.

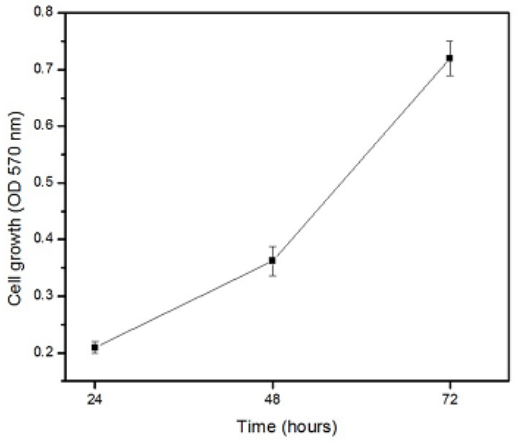

Figure 5. Cell growth curve of SKBR3 cancer cells

Table 2. Comparison of the experimental and simulated results

Simulation results (Gy)	Experimental results (Gy)
0.92	1
1.85	2
2.77	3
3.70	4
4.61	5
5.54	6

Survival fraction curves and radiosensitivity parameters

The cell survival fractions against radiation dose are illustrated on a log-linear scale (Figure 6). In Figure 6, the experimental data (mean values) are shown as points (with standard deviations) and the linear quadratic model fitted to the data are displayed as lines. The α, β, and α/β parameters obtained from LQ model fit were 0.156±0.027 Gy^{-1}, 0.026±0.007 Gy^{-2}, and 6.0 for 6 MV X-rays and 0.162±0.028 Gy^{-1}, 0.028±0.007 Gy^{-2}, and 5.8 for ^{60}Co gamma radiation, respectively. The calculation of the radiobiological parameters, namely α and β, was performed for the value of DRF that was equal to unit (G=1), since the temperature of the culture medium was kept at 4°C during the irradiation.

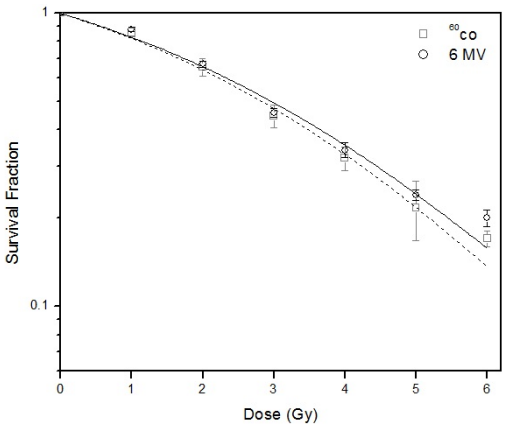

Figure 6. Survival fraction of SKBR3 cancer cells as a function of absorbed dose; radiation beams were 6 MV (dose rate of 1 Gy/min) ○— and ^{60}Co (dose rate of 0.7 Gy/min) □.... The symbols represent experimental data (including standard deviation in each point) and the lines are the LQ model fitted to the data.

Table 3 also illustrates a comparison between the radiobiological parameters of α, β, and α/β obtained in the present study with those reported by the previous studies for different breast cancer cell lines. This comparison revealed a wide variation range of radiobiological parameters for different regimes.

Relative biological effectiveness

It is completely known that when similar doses of different types of radiation are investigated, unequal biological effects are produced. In the present study, the RBE value was evaluated to compare the effect of various types of radiations. Average RBE value for the 6 MV (1 Gy/min) radiation, using ^{60}Co (0.7 Gy/min) as a reference radiation was calculated based on the survival data obtained from SKBR3 cells (Figure 7).

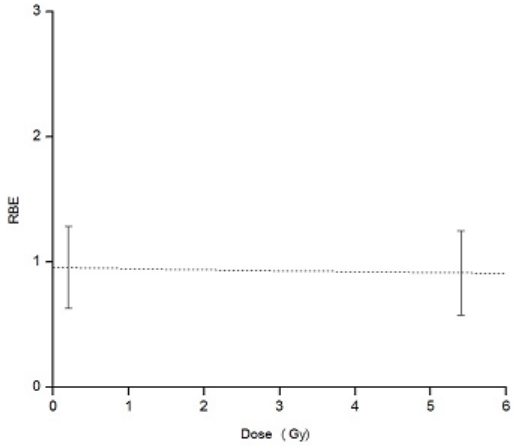

Figure 7. Average relative biological effectiveness value as the function of radiated dose for 6 MV photon energy (dose rate of 1 Gy/min) with ^{60}Co (dose rate of 0.7 Gy/min) as a reference radiation quality. Standard deviation values shown for doses under 1 Gy and over 5 Gy are representative for low doses (D≫D2) and high doses (D≪D2), respectively.

Table 3. Comparison of values of radiobiological parameters, namely α, β, and α/β, obtained from in vitro experiments for different human breast cancer cell lines in this study with those reported in the literature

Study	Subtype	ER	PR	HER2	Cell line	Radiation modality	Dose rate (Gy/min)	α (Gy-1)	β (Gy-2)	α/β
This study	HER2+	-	-	+	SKBR3	^{60}Co	0.7	0.162±0.028	0.028±0.007	5.8
This study	HER2+	-	-	+	SKBR3	6 MV	1	0.156±0.027	0.026±0.007	6
Ref. [5]	Luminal HER2-	+	+	-	T47D	6 MV	6	0.12±0.07	0.05±0.01	2.2
Ref. [5]		+	+	-		6 MV	0.2	0.12±0.08	0.06±0.01	2.4
Ref. [5]		+	+	-		380 kV	0.2	0.27±0.09	0.07±0.02	3.8
Ref. [5]		+	+	-		50 kV	0.2	0.38±0.05	0.07±0.01	5.2
Ref. [5]	Luminal HER2-	+	+	-	MCF7	6 MV	6	0.39±0.13	0.03±0.02	15
Ref. [5]		+	+	-		6 MV	0.2	0.33±0.04	0.03±0.01	11
Ref. [5]		+	+	-		380 kV	0.2	0.53±0.15	0.05±0.04	10
Ref. [5]		+	+	-		50 kV	0.2	0.74±0.09	0.008±0.001	96
Ref. [21]	HER2+	-	-	+	SKBR3	300 kV	-	0.274	0.035	7.8
Ref. [22]	Xenograft line				HX99	x-rays	1.5	0.2	0.05	4
Ref. [23]	Triple negative	-	-	-	MDA-MB-231	^{137}Cs	0.5	0.187±0.02	0.041±0.005	4.6
Ref. [24]	Triple negative	-	-	-	OCUB-M	200 kV	-	0.28	0.044	6.4

There were only small differences among the studied radiation qualities in terms of the cell survival. Due to the very small differences between the cell survival fractions for these radiation beams, the RBE values were calculated using the complete cell survival curve for a more precise estimation. Average RBE values were about 0.91-0.96 and increased with the reduction of doses.

Discussion

Survival fraction curves

As expected for low LET radiation sources (e.g., X- and γ-rays), a semi-logarithmic plot of survival fraction versus dose yielded a downward bending curve. As a general tendency, it can be observed that the cell survival fraction for photons with higher energy (6 MV x-rays with an average energy of 2 MeV) was slightly larger than that for photons with lower energy (^{60}Co γ-rays with an average energy of 1.25 MeV). The overestimation of survival is probable since there could be alive cells showing metabolic activity, but exhibiting rather poor or no cell proliferation in the survival outcome of this assay as explained by Buch et al. [11].

Comparison of radiosensitivity parameters with previously published data

In Table 3, several studies were selected to compare LQ radiosensitivity parameters for breast cancer cells using different regimes (e.g., radiotherapy modality and/or different dose rates with different delivered doses). This comparison showed a large disagreement among the available data, which can be due to several factors, such as various energies of photons, radiation modality, and dose rate.

According to this comparison, 50 kV photon radiations induced more efficient cell killing than the same dose given with 380 kV and 6 MV photon radiations. Considering the fact that there is no strong correlation between photon energies and cell survival fractions, the effect of different dose rates, which cause various sublethal repair time should be investigated.

Advantage of the current radiosensitivity parameters

The in vitro experiments in this study were performed at a temperature of 4°C. At this temperature, the sublethal repair process could be ignored. Therefore, the estimated radiobiological parameters in this study would serve as constant parameters. These parameters could be used in combination with different dose reduction factors (G values) for any given amount of dose rate. Therefore, the parameters obtained in this study could be utilized for the incorporation of biological effects into treatment plans and production of isoeffective dose curves. This facilitates the achievement of further improved TPS utilized in APBI, and thereby more effective treatments.

Conclusion

In this study, the radiobiological parameters, namely α and β, were estimated for SKBR3 breast cancer cells for the first time in a condition that no sublethal damage repair was allowed (G=1). Therefore, the estimated β parameter was independent of dose rate and was validated to be used in the TPS during APBI. The α and α/β parameters obtained in the present study were also compared with those obtained by the previous *in vitro* experiments for different breast cancer cell lines. The RBE values were obtained based on dose

and radiobiological parameters ratio (α/β) for SF endpoint. As the findings indicated, RBE value had no strong correlation with dose.

Acknowledgment

This study was supported by grant no. 33248, 6/2/2015 from the Deputy of Research and Technology of Ferdowsi University of Mashhad, Mashhad, Iran. Hereby, we acknowledge that parts of this computation were performed in the High Performance Computing Center of Ferdowsi University of Mashhad. We would like to thank the technical staff of the Elekta Compact accelerator and Theratron 780 ^{60}Co at the Shohada-e-Tajrish Hospital for their assistance during photon irradiation.

References

1. Smith BD, Arthur DW, Buchholz TA, Haffty BG, Hahn CA, Hardenbergh PH, et al. Accelerated partial breast irradiation consensus statement from the American Society for Radiation Oncology (ASTRO). Int J Radiat Oncol Biol Phys. 2009 Jul 15;74(4):987-1001.
2. Cox JA, Swanson TA. Current modalities of accelerated partial breast irradiation. Nat Rev Clin Oncol. 2013 Jun 1;10(6):344-56.
3. Tokatlı F, Dincer M. Breast-Conserving Therapy: Hypofractionated and Conventional Whole-Breast Irradiation and Accelerated Partial-Breast Irradiation. In: Aydiner A, Igci A, Soran A editors. Breast Disease: Management and Therapies. 1st ed. Switzerland: Springer International Publishing. 2016; 233-47.
4. Ruiz de Almodóvar JM, Núñez MI, McMillan TJ, Olea N, Mort C, Villalobos M, et al. Initial radiation-induced DNA damage in human tumour cell lines: a correlation with intrinsic cellular radiosensitivity. Br J Cancer. 1994 Mar 1;69(3):457-62.
5. Marthinsen AB, Gisetstad R, Danielsen S, Frengen J, Strickert T, Lundgren S. Relative biological effectiveness of photon energies used in brachytherapy and intraoperative radiotherapy techniques for two breast cancer cell lines. Acta Oncol. 2010 Nov 1;49(8):1261-8.
6. Steel GG, Deacon JM, Duchesne GM, Horwich A, Kelland LR, Peacock JH. The dose-rate effect in human tumour cells. Radiother Oncol. 1987 Aug 1;9(4):299-310.
7. Matthews JH, Meeker BE, Chapman JD. Response of human tumor cell lines in vitro to fractionated irradiation. Int J Radiat Oncol Biol Phys. 1989 Jan 1;16(1):133-8.
8. Guerrero M, Li XA. Analysis of a large number of clinical studies for breast cancer radiotherapy: estimation of radiobiological parameters for treatment planning. Phys Med Biol. 2003 Sep 30;48(20):3307.
9. Trialists' Group TS. The UK Standardisation of Breast Radiotherapy (START) Trial B of radiotherapy hypofractionation for treatment of early breast cancer: a randomised trial. The Lancet. 2008 Apr 4;371(9618):1098-107.
10. Ray M, Tan AW, Mangunkusumo AE, Lim DK. Confocal Microscopy of Cornea. In: Ng E. Y. K, Acharya U. R, Rangayyan R. M, Suri J. S editors. Ophthalmological Imaging and Applications, 1st ed. Florida: CRC Press; 2014; 195-214.
11. Buch K, Peters T, Nawroth T, Sänger M, Schmidberger H, Langguth P. Determination of cell survival after irradiation via clonogenic assay versus multiple MTT Assay-A comparative study. Radiat Oncol. 2012 Jan 3;7(1):1.
12. Andreo P, Burns D T, Hohlfeld K, Huq M S, Kanai T, Laitano F, et al. Absorbed dose determination in external beam radiotherapy: An International Code of Practice for dosimetry based on standards of absorbed dose to water. 1st ed. Vienna: IAEA Technical Report Series No. 398; 2000.
13. Scott BR, Hutt J, Lin Y, Padilla MT, Gott KM, Potter CA. Biological microdosimetry based on radiation cytotoxicity data. Radiat Prot Dosimetry. 2012 Aug 5;153(4):417-24.
14. Kloss FR, Singh S, Hächl O, Rentenberger J, Auberger T, Kraft A, et al. BMP-2 immobilized on nanocrystalline diamond-coated titanium screws; demonstration of osteoinductive properties in irradiated bone. Head Neck. 2013 Feb 1;35(2):235-41.
15. Calipel A, Lux AL, Guérin S, Lefaix JL, Laurent C, Bernaudin M, et al. Differential Radiosensitivity of Uveal Melanoma Cell Lines After X-rays or Carbon Ions Radiation. Invest Ophthalmol Vis Sci. 2015 May 1;56(5):3085-94.
16. Dale, R G, Fowler J F. Radiation repair mechanisms. In: Dale R G, Jones B editors. Radiobiological modelling in radiation oncology, 1st ed. London, UK: British Inst of Radiology; 2007; 97-105.
17. Menzel HG, Wambersie A, Pihet P. The clinical RBE and microdosimetric characterization of radiation quality in fast neutron therapy. Acta Oncol. 1994 Jan 1;33(3):251-9.
18. Collins AR. The comet assay for DNA damage and repair: Mol Biotechnol. 2004 Mar 1;26(3):249.
19. Collins AR, Dobson VL, Dusinská M, Kennedy G, Stětina R. The comet assay: what can it really tell us?. Mutat Res. 1997 Apr 29;375(2):183-93.
20. Mozdarani H, Nasirian B, Haeri SA. In vivo gamma-rays induced initial DNA damage and the effect of famotidine in mouse leukocytes as assayed by the alkaline comet assay. J Radiat Res. 2007 Feb 14;48(2):129-34.
21. Lechtman ES. A Monte Carlo-based model of gold nanoparticle radiosensitization. (Doctoral dissertation), University of Toronto; 2013. Retrieved from https://tspace.library.utoronto.ca/handle/1807/43632.
22. Kelland LR, Steel GG. Dose-rate effects in the radiation response of four human tumour xenografts. Radiotherapy and oncology: Radiother Oncol. 1986 Nov 1;7(3):259-68.
23. Hobbs RF, Howell RW, Song H, Baechler S, Sgouros G. Redefining relative biological effectiveness in the context of the EQDX formalism: Implications for alpha-particle emitter therapy. Radiat Res. 2013 Dec 30;181(1):90-8.
24. Matsuzaki H, Miyamoto T, Miyazawa Y, Okazumi S, Koide Y, Isono K. Biological Effects of Heavy Ion Beam on Human Breast Cancers. Breast Cancer. 1998 Jul 1;5(3):261.