Jurnal Sains Nuklear Malaysia, 2018, 30(2): 15 – 22
ISSN: 2232-0946

RADIOBIOLOGICAL EVALUATION OF CELLS IRRADIATED WITH PHOTON AND ELECTRON BEAMS RADIOTHERAPY USING LINEAR QUADRATIC AND MULTI-TARGET MODEL

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ABSTRACT

Radiobiological model such as linear quadratic (LQ) is widely used in radiotherapy to predict the biophysical response of the tumour cell to the radiation. In clinical radiotherapy, LQ model is widely employed to plan treatment delivery and fractionation. Nevertheless, LQ model might not provide accurate prediction for high dose rate treatment. This study investigates the radiation cell survival responses using LQ model and alternative Multi-Target (MT) model. The experimental works were conducted in-vitro using HeLa cells that were irradiated using photon and electron beams of different energy. Cells irradiation were performed in full scatter condition and exposed to radiation doses ranges from 1 to 10 Gy. Clonogenic assay is used as an endpoint to obtain the cell survival curves which later be fitted with LQ and MT model. The results demonstrate that MT model produce the fitting curves that are closed to the experimental data compare to LQ model especially at high doses. Parameter analysis from both models indicates more biological damage inflicted by high energy electron beam. Correlation between the experimental cell survival data and radiobiological model analysis suggesting that alternative radiobiological model such as MT model could be applied in analysing cells' radiation survival and damage in clinical radiotherapy.

Keywords: Linear quadratic model, multi-target model, radiation cell survival, radiobiology

INTRODUCTION

The cell survival curve was used in the research done by Puck and Marcus (1956) to describe the effects of high energy irradiation on the reproductive capacity of single HeLa cells by plotting the graph of number of cells colony versus dose. According to the graph plotted by Puck and Marcus, it defines the relationship between the radiation dose and the proportion of cells that survive (Puck and Marcus, 1956). In addition, the cell culture assay technique was recommended by Markis et al. (2012) in order to obtain the cell survival curve and the surviving fraction can be calculated by counting the cell colony form after staining. There are several models used to analysis the cell survival curve such as linear quadratic (LQ) and multi-target (MT) model. Classical MT model presents a straight line at high doses, which is not supported by the mechanism of the underlying radiobiological processes but it is still valuable because MT model fits the empirical data well, especially in the high-dose range. Iwata et al. (2012) states that conversion with the MT model is not easy in clinical practice since there are many parameters which generally cannot be determined. Later, LQ model was proposed by Chadwick and Leenhouts in 1981 in order to correct the deficiencies of the MT model (Ballarani, 2010; Chadwick and Leenhouts, 1981). Fertil and Malaise (1985) found that human cell survival curves could be better described by the LQ model compare to

MT model. The LQ model describes low α-value indicates resistence in the low dose range and is associated with high P-value. The exponential survival curve shows the high radiosensitivity of cells while shouldered survival curve represents the poor radiosensitivity. There are several studies that compare different models to analyse the cell survival curves. One of the studies comparing the different model was one by Iwata et al. (2012). From the study, it shows that MT models seem to be more reliable than the LQ model at 6 Gy or higher single doses. This result may due to the characteristics of the two models where at the high-dose range the data can be approximated by linear regression. The LQ model may only applicable to fractional doses of 5 Gy or less. Even though MT model is a basic and classical linear model, it still fits the empirical data well at high doses compared to the LQ model (Iwata et al., 2012). Besides that, Fertil and Malaise (1985) found that parameters used by MT model that are n and D_0 do not accurately describe the differences in radiosensitivity that are a feature of the initial part of the survival curves. In the LQ model, the use of the parameter α not only allows the differentiation between different cell lines, but also leads to consistency between different studies of a single cell line, as in the case of HeLa cells (Fertil and Malaise, 1985).

In clinical radiotherapy, the determination of the doses and treatment schemes were planned with the guidance of LQ model. The LQ model work very well for conventional radiotherapy consist of low doses schemes. However, applicability of LQ model for advanced radiotherapy such as stereotactic radiosurgery, stereotactic radiotherapy and high dose rate brachytherapy that used high dose fraction is limited. In this study, the applicability of the MT model in comparison to LQ model was investigated and the both of models' parameters were analysed to find correlation with experimental data.

MATERIALS AND METHODS

HeLa Cell Lines Preparation

HeLa (ATCC® CCL-2™) cell lines were prepared in Dulbecco's Modified Eagle's Medium (DMEM) which was supplemented with 10% FBS and a 100 unit/mL penicillin-streptomycin (Gibco, Life Technologies, CA, U.S.A). The cells were grown in 37^{o}C and 5% CO_2 humidified atmosphere until confluence and harvested using 0.25% Trypsin-EDTA (Gibco, Life Technologies, CA, U.S.A).

HeLa Cells Irradiation Setup

The cell samples were prepared in suspension in 0.5 ml PCR tube (Greiner Bio-One, Austria) which contains approximately 1000 cells. Irradiation was carried out using clinical photon and electron beams of different energies using Primus Linear Accelerator (Siemens Healthcare, USA) at the Nuclear Medicine, Radiotherapy and Oncology Department, Hospital Universiti Sains Malaysia. The cell samples were set up on a plastic water phantom at source to surface distance (SSD) of 100 cm with 10 x 10 cm^2 field size. Bolus was placed on top of cell samples as a build-up so that the maximum prescribed dose was delivered to the cells. Irradiations were done in single fraction with constant dose rate of 100 MU/min with radiation doses ranging from 0 to 10 Gy. The irradiation setups are shown in Figure 1 and 2. Irradiated cells were incubated for 10 days to observe the colony formation.

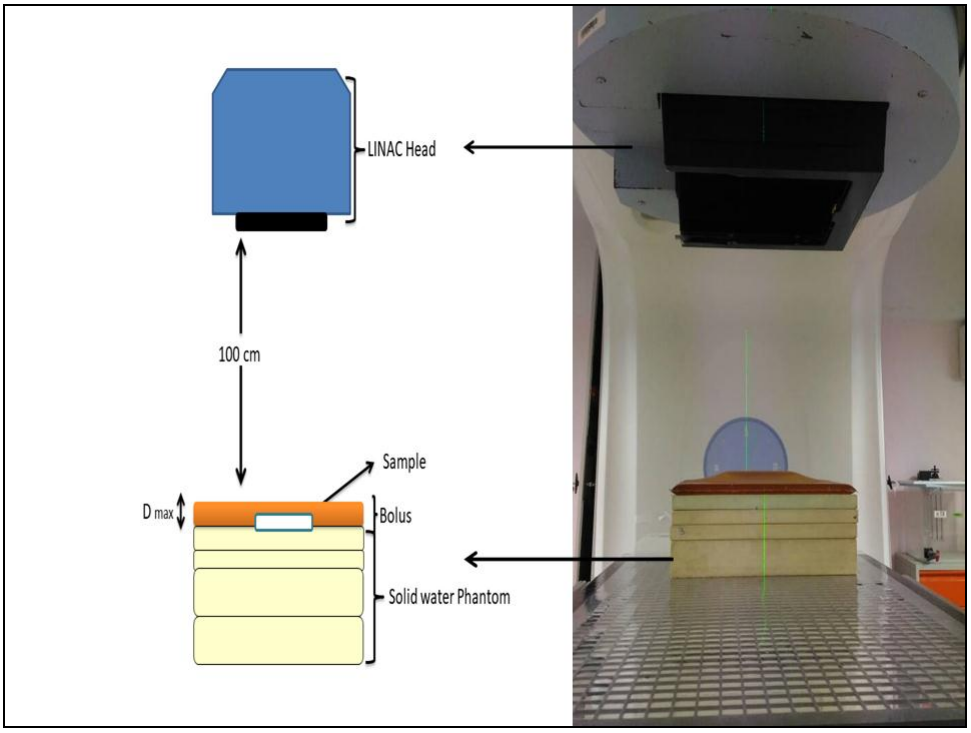

Figure 1: The irradiation setup for photon beams

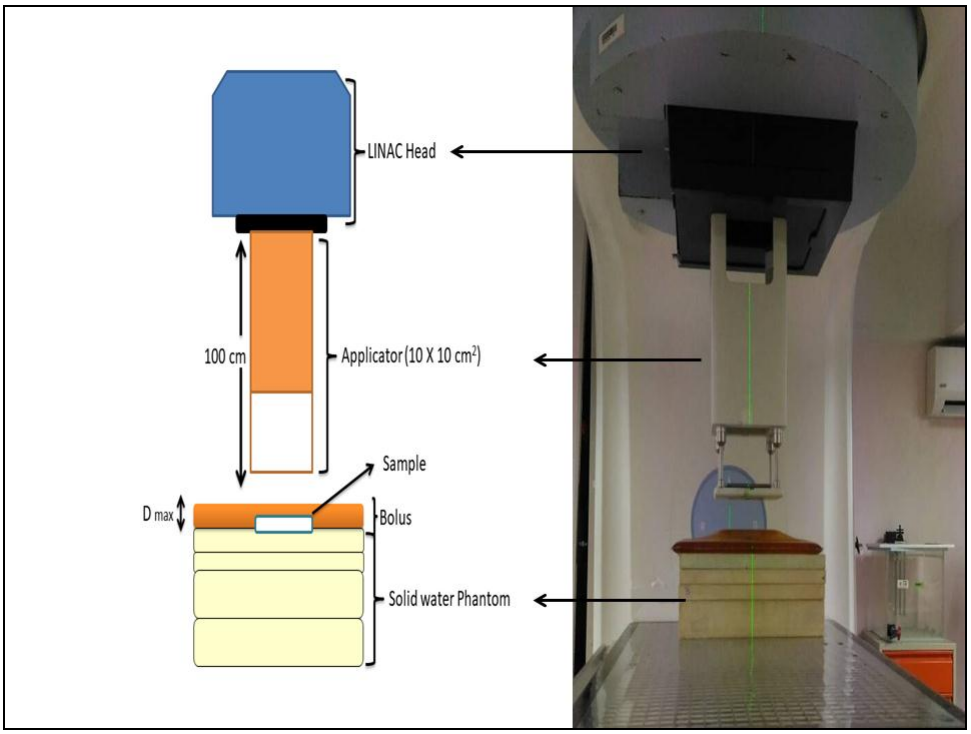

Figure 2: The irradiation setup for electron beams

Clonogenic Assay

After 10 days of incubation, the irradiated and non-irradiated (control i.e: 0 Gy) cell samples were washed from the cell media using 0.5 mL of PBS. The cells were then fixed using 0.5 mL ice cold methanol for 15 minutes. The fixed cells were stained with crystal violet for 30 minutes and were rinsed gently using tap water. After rinsing, the stained cells were let to dry before counting the cells colony. The visible cell colonies were counted using microscope and analyzed in form of cell survival fraction data using OriginPro 7.5 software (OriginLab Corporation, Northampton, MA, USA).

Radiobiological Analysis

The linear quadratic (LQ) and multi-target (MT) model were fitted to the experimental data point using OriginPro 9.0 software. The models equations are shown in equation 1 and 2. Explainations on the equation's parameters are also described. Analyses of the parameters value from the survival curves generated from each model were also performed.

$$\text{Linear quadratic model: } S = \exp^{-(\alpha D + \beta D2)} \quad \text{..... (1)}$$

Where α and β are dependents, and D is the absorbed dose. In the other word, the LQ model assumes that S is made of two terms: a linear term αD and a quadratic term, βD^2 (Joiner and Kogel, 2009). The molecular and cellular justifications are as follows:

i. A lethal lesion can be thought of as an unrepaired DNA double strand break (DSB) that leads to chromosome breaks, which in turn lead to cell death.

ii. A lethal lesion can be produced either by single radiation track (which is proportional to dose, αD) or by interactions of two or more less severe DNA lesions (sublethal lesions) generated by two separate radiation tracks (which is proportional to the dose square, βD^2) (Wang, 2010).

The second radiobiological model used is multi-target model. Data were fitted according to the model equation expression that also describes the relationship between the cell survival and irradiation dose:

$$\text{Multi-Target Model: } S = 1 - (1 - \exp^{(-D/D_o)})^n \quad \text{.....(2)}$$

Where D is the dose in Gray, D_o is parameter that determines the final slope of the survival curve, and n is the y-intercept of the asymptote (Joiner and Kogel, 2009). The MT model is a basic and classical linear model which fits the empirical data well at high dose compared to the LQ model (Iwata et al., 2012; Nomiya, 2013).

Statistical Analysis

The data are expressed as the mean ± standard deviation of three samples. One way analysis of variance (ANOVA) was used to determine the significance of the difference between control and experimental group followed by post-hoc analysis mean comparison using Bonferroni's test. A

difference was considered to be statistically significant when $p < 0.05$. Statistical analysis was performed with the OriginPro 9.2 software (OriginLab Corporation, Northampton, MA, USA).

RESULTS AND DISCUSSIONS

In this study, cell survival at different radiation doses for different energy of clinical photon and electron beams were obtained. Photon and electron beams were used in this study because both of the beams are common radiotherapy beams used to treat cancer patients. The experimental cell survival data were then fitted with LQ and MT model. Figure 3 shows the cell survival curves for 6 and 10 MV photon beams fitted with LQ and MT model. The MT model is found to fit the experimental data better compare to the LQ model for 6 MV photon beam. Meanwhile the cell survival curves for 10 MV photon beam generated with LQ and MT model are almost identical.

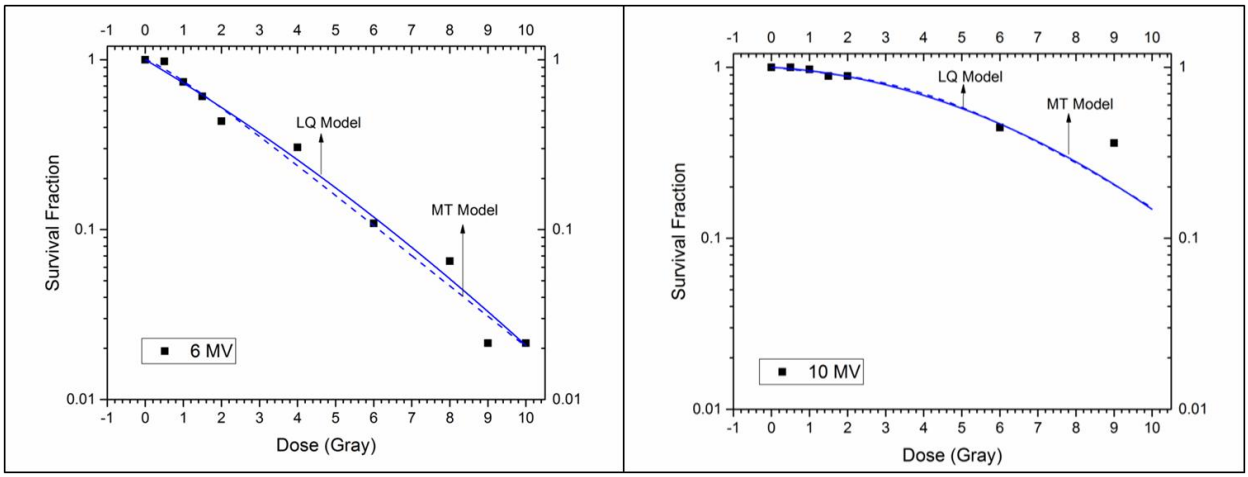

Figure 3: The cell survival curves for 6 and 10 MV photon beams

Figure 4 shows the graph for 6 and 15 MeV electron beams fitted with LQ and MT model. The fitting curve of LQ and MT models were found to fit the experimental data better at low dose compare to high dose. The fitting curves for LQ seem to fit the experimental data better than MT model for 6 MeV electron beams. Meanwhile for 15 MeV electron beams, the results are contradictory in which the MT model fitted the experimental data better at low and high dose compare to LQ model.

In general, survival fractions generated by MT model are slightly higher compare to LQ model for dose ranges from 0.5 Gy to 1.5 Gy. At higher dose up to 10 Gy, LQ model indicate higher survival fraction compare to MT model. LQ model fitted the cell survival data well for photon beams and 6 MeV electron beams except at high dose where MT model is more suitable.

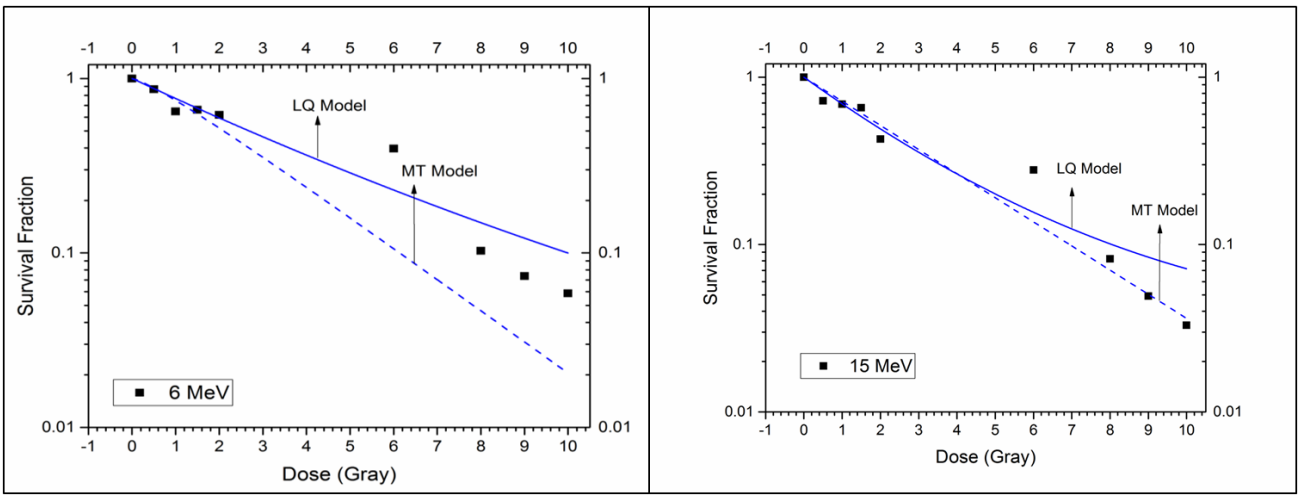

Figure 4: The cell survival curves for 6 and 15 MeV electron beams

Radiobiological analysis of the cell survival based on the LQ model parameters is shown Table 1. Parameter for α (Gy^{-1}) represents single radiation hit to the cells where the cells are presume to be unable to repair themselves after the radiation exposure. Value β (Gy^{-2}) represent double radiation hits to the cells where it can repair after the damage caused by the radiation (Joiner and Kogel, 2009). Based on the results in Table 1, both 6 MV and 10 MV photon beams shows the value of α (Gy^{-1}) is higher than β (Gy^{-2}). Therefore, it indicates that more cells death due to single radiation hit to the cells. The α (Gy^{-1}) value of 6 MV photon beam is higher than 10 MV photon beam while β (Gy^{-2}) value for 10 MV photon beam is higher than 6 MV photon beam. This indicate the cells that were irradiated with 10 MV photon beam has higher tendency to be repair compare to 6 MV photon beam (Ballarini, 2010). Comparison of parameters value of α (Gy^{-1}) and β (Gy^{-2}) between 6 MV and 10 MV photon beams demonstrate that low energy kills more cells compare to high energy. However for electron beams, the value α (Gy^{-1}) of 15 MeV is higher than 6 MeV, and much more impressively higher compare to photon beams. The LQ model parameters analysis signifies the experimental cell survival obtained to the single radiation hits as the main cause of cell death.

Table 1: The parameters value from Linear Quadratic model

Beam Types	**Energies**	**α (Gy^{-1})(SE)**	**β (Gy^{-2})(SE)**
Photon Beams	6 MV	0.3072 (± 0.05)	0.0079 (± 0.01)
	10 MV	0.0305 (± 0.04)	0.0160 (± 0.01)
Electron Beams	6 MeV	0.2677 (± 0.06)	-0.0037 (± 0.01)
	15 MeV	0.3790 (± 0.06)	-0.0115 (± 0.01)

Table 2 shows the parameters value for MT model. The value of D_1 presents the initial slope of the curve due to single event killing while D_0 presents the value of final slope of the curve due to multiple event killing (Joiner and Kogel, 2009). The n value is the extrapolation number. MT model parameter analysis for 15 MeV electron beam shows the highest values of D_0 compare to other energies used. This shows that multiple event killing have greatest effect to cells death. Similar to

LQ model analysis, electron beam causing more biological damage compare to photon beam as indicate by lower value of D_0 for photon beams (Ballarini, 2010).

Table 2: The parameters value from Multi-Target Model

Beam Types	**Energies**	**D_1**	**D_0**	**n**
Photon Beams	6 MV	3.7549E19 (± 0)	2.4239 (± 0.42)	1.2709 (± 0.25)
	10 MV	18.1207 (± 16.76)	3.3999 (± 2.12)	5.6001 (± 9.53)
Electron Beams	6 MeV	3.7549E19 (± 0)	2.4239 (± 0.42)	1.2709 (± 0.25)
	15 MeV	3.0136 (± 0.41)	4022.76 (± 0)	981.2117 (± 0)

Understanding basic cell's responses to these clinical radiotherapy beams are extremely crucial to ensure efficient cancer treatment. This study fundamentally approaches to simulate and understand cancer cells' response under radiation therapy using radiobiological model. Implementation of radiobiological modelling for planning radiotherapy treatment helped clinicians to understand better the outcome of the treatment while correlate with dosimetric aspects (Iwata et al., 2012). The mechanism of radiobiological systematic method formed a bridge of physical quantity (dose deposition in tissue) and the actual radiation-activated cell death in clinical radiotherapy practices (Zhang et al., 2017).

The LQ model has been the most useful model for treating tumours with conventional fractionated radiotherapy. The validity of the LQ model in calculating dose fraction for high dose radiotherapy schemes for advanced radiotherapy techniques has been intensively debated (Chang et al., 2013). Alternative model have been introduced to overcome this limitation (Kehwar, 2017). In this study, preliminary results on cells' response to different types of radiotherapy beams and evaluated by the LQ and MT model indicate the importance of radiobiological model for radiotherapy outcome prediction. More investigation is required to understand the effects of dose fractionation, isoeffect doses and different radiotherapy techniques.

CONCLUSIONS

In conclusion, the MT model was found to be close to experimental cell survival and generate better fitting curve compare to the LQ model especially at high dose. The analysis from both models parameter also present correlation with cell survival data and could be apply to predict the cells' response to radiotherapy. Therefore alternative radiobiological model such as MT model is useful for interpreting radiation response over different advanced radiotherapy techniques.

ACKNOWLEDGEMENTS

This study was supported by FRGS grant 203/PPSK/6171160. We gratefully acknowledge the use of the Medical Linear Accelerator in Nuclear Medicine, Radiotherapy and Oncology Department at Universiti Sains Malaysia Hospital.

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