HIROSAKI UNIVERSITY

Radiation Environment and Medicine 2020 Vol.9, No.1 1–6

REM
Radiat Environ Med

Regular Article

A Comparison of Radiobiological Response in Cells Exposed to Low LET Radiation with Different Beam Energies

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Received 2 March 2019; revised 21 June 2019; accepted 31 July 2019

There is conflicting evidence in the literature on biological effects between different low LET radiations. The aim of this study was to investigate the impact of different low LET radiations on radiobiological response.

Chromosomal radiosensitivity was measured in human lymphocytes from healthy donors using the G2 chromosomal radiosensitivity assay. Radiation induced mitotic inhibition was also measured.

Cells were irradiated to doses of 0.05 and 0.5 Gy using a Cobalt 60 teletherapy unit and a Linac operating at 6 MV (photons) and 12 MeV (electrons). As expected, a dose response was observed with increasing dose resulting in greater response. No statistically significant differences were observed, however, with different beam energies. Our study shows no statistically significant difference in radiobiological response with beam energy as measured using G2 chromosomal radiosensitivity and radiation induced mitotic inhibition as endpoints.

Key words: low LET radiation, photons, electrons, chromosomal aberrations, mitotic inhibition

1. Introduction

It is well known that biological effects differ depending on cell and tissue type, dose and linear energy transfer (LET)[1)]. Generally, higher relative biological effectiveness (RBE) is observed with increasing LET[2-6)], but at very high LET, the RBE begins to change, as shown by Barendsen[7)]. Many studies have compared DNA double strand breaks, reproductive cell death and chromosome aberrations in cells irradiated in vitro with sparsely ionizing radiation of low LET or densely ionizing radiation of higher LET[8-13)]. A number of radiobiological studies have also shown an increase in RBE with decreasing energy for low LET radiations, electrons and photons (reviewed in[14-16)]).

Chromosome damage has been reported in the literature as an effective endpoint for assessing radiation damage. The first study which demonstrated radiation damage to chromosomes using Trudescantia microspores,

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also showed a lower RBE of Cobalt 60 gamma radiation compared to 250 kVp X-rays[17], most likely as a result of a slightly higher RBE of 250 kVp X-rays as shown by Fowler[18]. Subsequent studies on human lymphocytes have shown that relatively high energy low LET radiation, such as gamma radiation, is several times less effective than relatively low energy low LET radiation, such as low energy X radiation[15, 19-23]. Despite evidence from in vitro radiobiological data that the RBE of high energy low LET radiation is less than that of low energy low LET radiation, there is limited epidemiological data to show that cancer risk decreases as radiation energy increases for low LET radiations (reviewed by[15]). Despite the radiobiological data suggesting that low LET radiations of different energy show different RBE, for radiation protection, the ICRP 2007 recommendations retain a weighting factor of 1 for all low LET radiations[24]. However an ICRU 1978 report states that absorbed dose values for 100-300 kV X-rays should be multiplied by 1.18, and not 1, when comparing effects to MV X-rays[25]. A recent study showed cluster patterns of energy deposition sites to differ up to 15% between low photon energy brachytherapy sources (^{103}Pd and ^{125}I with mean photon energies of 0.021 and 0.028 MeV) and less than 2% for high photon energy (^{192}Ir and ^{137}Cs with mean photon energies of 0.36 and 0.615 MeV) brachytherapy sources with respect to ^{60}Co[26] which correlated with reported RBE values for double strand break yields[27].

As this area is still unclear, further investigation is warranted. This present study aimed to investigate the impact of different low LET radiations and dose on radiobiological response of human lymphocytes. The G2 chromosomal radiosensitivity assay, or G2 assay, was employed for this study as increased chromosomal aberrations have been linked to increased individual radiosensitivity and predisposition to cancer[28-30]. It is a cytogenetic based assay where whole blood samples are cultured and irradiated in the G2 phase. Chromosomal damage is then quantified at metaphase as chromatid gaps and breaks. Although the assay is typically carried out using a 0.5 Gy dose point, an additional low dose point of 0.05 Gy was also included in the present study to investigate the effect of dose as well as beam energy. Radiation induced mitotic inhibition was also investigated as a measure of checkpoint response[31, 32].

2. Methods

2.1. Donor information and Ethics

Blood samples were donated from 20 volunteers at our Institute. The ratio of donors was 50% female to 50% male, with an age range from 22-43 years. 20 ml of circulating peripheral blood was extracted into heparinised vacutainers (Sarstedt, Ireland). Ethical approval for this study was granted by the DIT Research Ethics Committee (REC 15-32).

2.2. Whole blood culture

Whole blood cultures for each dose (0 Gy, 0.05 Gy and 0.5 Gy) were set up in triplicate flasks using 2 ml of whole blood and 18 ml of pre–warmed (37℃) pre–gassed (5% CO_2) RPMI1640 medium (Sigma, Ireland) supplemented with 12.5% FBS (Sigma, Ireland), 2 mM L-glutamine (Sigma, Ireland) and 0.2 ml (45mg Phytohaemagglutinin (PHA) (PAA Laboratories, Fisher Scientific, Ireland). Cultures were incubated at 37℃ with 5% CO_2 for 72 hours prior to radiation exposure. Whole blood cultures were stimulated with the mitogen PHA to induce cycling to increase lymphocyte number for analysis 72 hours later. 1 hour prior to irradiation a media change was carried out.

2.3. Irradiation

At 72 hours, whole blood cultures were irradiated to low doses of 0.05 Gy and 0.5 Gy using three different radiation sources at St. Luke's Hospital (Dublin, Ireland). The first set was irradiated using the Cobalt 60 Teletherapy unit (Theratron 780 E, MDS Canada). The second set was exposed to a 6 MV photon beam and the third set to a 12 MeV electron beam produced by an Elekta Precise linear accelerator (LINAC).

Dosimetry was performed with Gafchromic film. The film was calibrated against a Farmer type ionization chamber (for 6 MV photons) and against a Roos parallel plate ionisation chamber (for 12 MeV electrons) using the triple channel dosimetry method[33]. The film was scanned using the single scan protocol[33] on an Epson Expression 10000 XL scanner using the recommended scanning resolution of 72 dpi in a 48-bit RGB format[34, 35].

Glass was placed over the calibration and test film during scanning to minimize 8 ringing artifacts. The film was analysed using FilmQAe Pro (Ashland Inc., USA).

2.4. Cytogenetics

Blood cultures were incubated at 37℃ post irradiation for 30 minutes and 200μl colcemid (1μg/ml stock in 20 ml giving a final concentration of 0.01μg/ml) (Gibco, BioSciences, Ireland) was then added for 60 minutes at 37℃ to arrest cells in the first metaphase (M phase) by inhibition of spindle formation. This allowed for visualisation of chromatid aberrations in those cells in M phase.

All samples were then placed in ice cold hypotonic solution (0.075 M KCl) for 20 mins on ice before centrifugation at 1400 rpm for 10 minutes. The extracted lymphocytes were fixed and washed twice in Carnoy's fixative (3:1 methanol:acetic acid, Lennox, Ireland). All cytogenetic procedures were carried at cool temperatures to delay chromatid repair mechanisms. Slides were pre-

cleaned in methanol 24 hours prior to use. The fixed lymphocyte suspension was dropped on to each slide in triplicate. Slides were briefly flamed over a Bunsen burner to fix cells onto the slide and stained with 3% Giemsa (Gibco, BioSciences, Ireland) prepared in pH 6.8 buffer (BDH Chemicals, VWR, Ireland) for 15 minutes, air dried and mounted in DPX (BDH Chemicals, VWR, Ireland).

All slides were coded and scored anonymously to first measure the mitotic index per donor sample and respective radiation-induced mitotic inhibition using a 10x objective lens. The mitotic index was used to indicate the percentage of cells arrested in the stage of mitosis at the time of sampling and was determined by counting the ratio of metaphase cells to interphase cells on each slide (counting 1000 total cells in total using a 40x objective lens). The radiation induced mitotic inhibition value was calculated from each donor sample by subtracting the dose (0.05 Gy and 0.5 Gy) mitotic index values from the corresponding control (0 Gy) mitotic index values representing the cell cycle checkpoint response.

Only well spread metaphases ($n = 46$) were analysed using a 100x objective lens for chromatid aberrations and any cases of polyploidy noted. Spontaneous aberrations (0 Gy) and radiation-induced (0.05 Gy and 0.5 Gy) chromatid aberrations per 100 metaphases were recorded. Chromatid aberrations were primarily chromatid gaps and breaks but additional rare chromatid aberrations were recorded, such as chromatid minutes, terminal deletions and inter-arm exchanges. The total number of aberrations per 100 metaphases scored in each sample constituted a G2 score, mostly consisting of chromatid gaps and breaks. The G2 score for the 0 Gy sample (spontaneous abberations) was subtracted from the G2 score of the irradiated samples 0.05 Gy and 0.5 Gy independently to determine a radiation-induced G2 score for each dose.

2.5. Statistical Analysis

The radiation induced G2 scores were compared per donor for each dose (0.05 Gy and 0.5 Gy) and for each radiation source. Radiosensitivity levels were determined by a calculated G2 radiosensitivity cut off point (90th percentile of G2 scores) at 0.5 Gy (31). This cut off value represents the value at which 90% of the observed population are found; so that donor radiation induced G2 scores exceeding this cut-off were deemed radiosensitive.

Inter-variation is a measure of reliability of the assay as it assigns a % variation between the group of individuals sampled. Intra-variation is a number of samples taken from one individual on different occasions. Both inter and intra variation analysis was done to assess reliability of the assay by calculating the coefficient of variation (CV) which is equal to the standard deviation divided by the mean and multiplied by 100.

Comparison of results for the different doses and energies used was performed using ANOVA. All statistical analysis was carried out using Graphpad Prism 5.

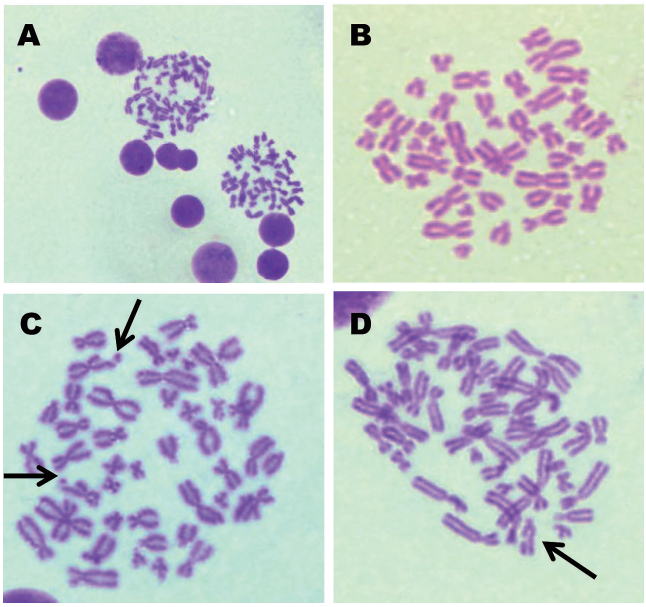

Fig. 1. Giemsa stained metaphase spreads showing examples of A) Interphase and Metaphase cells, B) 0 Gy Control metaphase with no aberrations, C) 0.5 Gy metaphase indicating chromatid breaks (black arrows) and D) 0.05 Gy metaphase indicating chromatid gap (black arrow). 100 metaphase spreads were analysed per radiation dose and donor and the G2 score comprising chromatid breaks and gaps was calculated.

3. Results

The total amount of radiation induced G2 chromatid damage is representative of the intrinsic radiosensitivity in each individual donor per dose and source. Examples of the types of aberrations recorded to give a G2 radiosensitivity score can be observed in Figure 1. A G2 radiosensitivity score was calculated by subtracting the 0 Gy control (non - irradiated spontaneous aberrations) from the irradiated G2 scores per donor.

Inter-individual variation was calculated based on G2 radiosensitivity scores from 20 donors which is performed as a measure of assay reproducibility. Inter-variation values of all sources fell below a value of 23% at 0.05 Gy and below 20% at 0.5 Gy (Table 1). Additionally intra–individual variation was calculated using one donor, sampled on 8 different occasions which fell below a value of 10.4% at 0.05 Gy and below a value of 12.9% for 0.5 Gy. Table 1 also shows the mean, standard deviation and coefficient of variation per dose and per source.

A radiosensitivity cut off value was defined by calculating the 90th percentile of each group at each dose (Table 1). This value represents a threshold below

Table 1. Summary statistics for radiation induced G2 scores for all beam sources at 0.05 Gy and 0.5 Gy. Mean, standard deviation, coefficient of variation (CV) and radiosensitivity cut offs (90th percentile) are shown

		^{60}Co	Linac Photon	Linac Electron
0.05 Gy	Mean	33	33	35
	Standard Deviation	7	8	7
	CV (%)	20	23	22
	Radiosensitivity cut off (90th percentile)	40	44	42
0.5 Gy	Mean	125	113	116
	Standard Deviation	22	18	23
	CV (%)	18	16	20
	Radiosensitivity cut off (90th percentile)	147	132	144

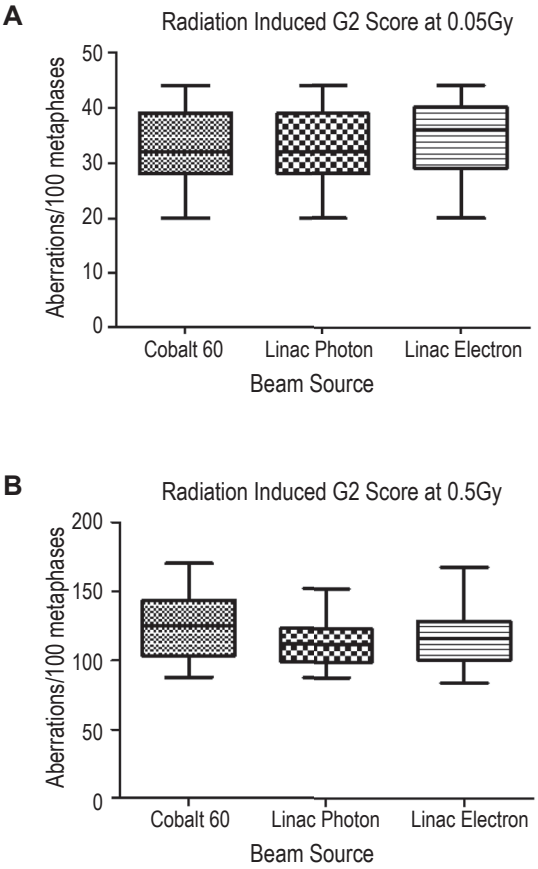

Fig. 2. G2 chromosomal radiosensitivity at low doses from clinical sources (Cobalt -60, Linac photon 6 MV and Linac electron 12 MeV). Boxplots show G2 chromosomal radiosensitivity scores from blood samples taken from 20 health donors irradiated to A) 0.05 Gy and B) 0.5 Gy. The spontaneous aberration yield from the 0 Gy control has been subtracted from each dose to give a radiation induced G2 radiosensitivity score. Error bars represent upper and lower values.

which 90% of the population reach. Donors who have radiosensitivity scores above this threshold are considered radiosensitive.

Distribution of G2 radiosensitivity scores are illustrated as boxplots in Figure 2 for each dose and each source. No statistically significant difference between radiation sources (Cobalt 60, linac photon and linac electron) was observed. A significant difference in G2 score between 0 Gy control and irradiated (0.05 Gy and 0.5 Gy) samples was observed ($P < 0.001$). In addition, a significant difference between each dose (0.05 Gy and 0.5 Gy) was observed ($P < 0.001$) with G2 scores increasing at least three fold. Comparison of G2 scores between radiation sources was performed using ANOVA. No statistically significant difference in G2 scores between sources was observed ($P > 0.05$).

Cell cycle checkpoint response was assessed by calculating a radiation induced mitotic inhibition value (MIn). MIn values per dose and radiation type are shown in Figure 3. Comparison of means showed that inhibition was observed in all donors with MIn values below 5%. MIn values between 0 Gy and each dose were significantly different ($P < 0.0001$) whereas MIn between 0.05 Gy and 0.5 Gy were not significantly different ($P = 0.2$). MIn values were compared between radiation source (Cobalt 60, Linac Photon and Linac Electron) using ANOVA. Inhibition was observed in all donors across all sources, but no statistically significant difference was observed between sources ($P > 0.05$).

4. Discussion

For radiation protection, all low LET radiations are assigned the same radiation weighting factor of one[24]. However, there is still considerable debate over the effectiveness of different low LET radiations and recent reviews have highlighted that low LET radiations of different energy show different RBE[14-16]. The present study was undertaken to compare radiobiological responses in human lymphocytes exposed to low LET radiation with different beam energies.

The results showed increased chromosomal radiosensitivity with increasing dose as observed in the overall increase in number of aberrations between 0.05 Gy and 0.5 Gy. Significant differences were observed between aberration yield in non-irradiated control samples and between each dose, with an increase in aberration yield between 0.05 Gy and 0.5 Gy. Similar results were observed for G2 scores with a maximum value of 44 at 0.05 Gy and a maximum value of 170 at 0.5 Gy. The radiosensitivity cut off values used in this study as indicated by the 90th percentile values (40 − 44 for 0.05 Gy and 132–147 for 0.5 Gy) were in line with previous published work for a 0.5 Gy dose. Scott *et al.*[36] and Riches *et al.*[37] defined 80 aberrations/100 metaphases as a radiosensitivity cut-off value, while Baria *et al.*[38] and Howe *et al.*[39] used 100 aberrations/100 metaphases and Baeyens *et al.*[40] used

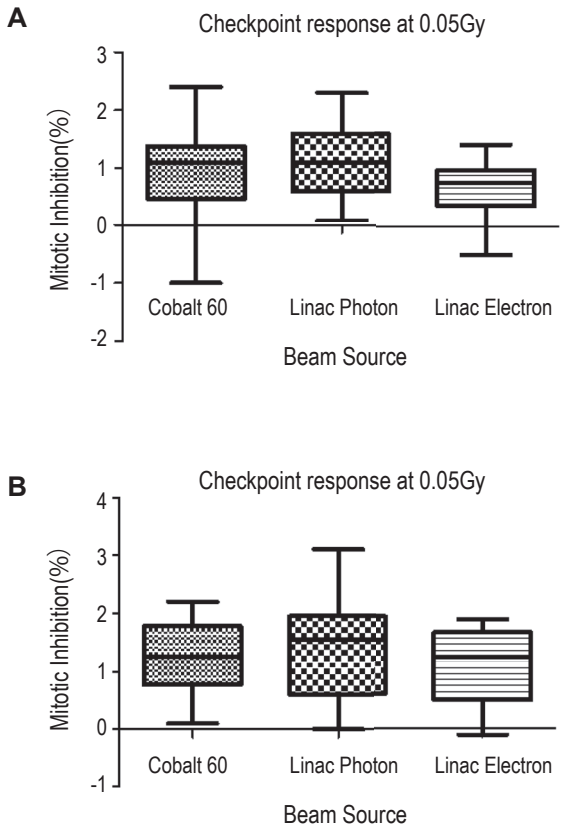

Fig. 3. Checkpoint response as calculated from radiation induced mitotic inhibition at A) 0.05 Gy and B) 0.5 Gy. Mitotic index values for each dose point were subtracted from the 0 Gy control to give a mitotic inhibition value. Data from Cobalt-60, Linac photon 6 MV and Linac electron 12 MeV are presented. Error bars represent upper and lower values.

129 aberrations/100 metaphases. The measure of inter variability which indicates the assay's reliability and reproducibility as indicated by the coefficient of variation (CV) values in table 1 were also in line with published values[41-43].

Checkpoint response was observed at 0.05 Gy and 0.5 Gy where the mitotic index decreased after irradiation and this agrees with previous studies indicating cell cycle checkpoint abrogation and blockade of mitotic entry in the cell cycle[31, 32].

The distribution of G2 scores and mitotic index values between sources showed similar minimum and maximum values and overlapping error bars demonstrating no significant difference in G2 radiosensitivity or mitotic inhibition between sources. Therefore regardless of beam source, the same effect was observed.

In summary, our study shows no statistically significant difference in radiobiological response with beam energy as measured using G2 radiosensitivity and mitotic inhibition as endpoints.

Acknowledgements

The authors acknowledge funding from Science Foundation Ireland, 11/RFP.1/BMT/3317, and the EU FP7 Network of Excellence DoReMi, Grant Number 249689.

Conflict of Interest

The authors declare that they have no conflict of interest.

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