A COMPARATIVE STUDY OF BIOLOGICAL EFFECTS OF ELECTRONS AND Co-60 GAMMA RAYS ON pBR322 PLASMID DNA

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Abstract

We investigate the damage caused by 6 – 15 MeV electrons to pBR322 plasmid DNA and compare the break yield to that of Co-60 gamma rays to develop an understanding of the mechanisms of electron-induced DNA damage. Plasmids were chosen to allow for observation of DNA damage in isolation – unlike cells, plasmids have no repair mechanisms, so any damage remains unrepaired. We outline the set-up, analysis and results of plasmid irradiation experiments carried out at the Dalton Cumbrian Facility (UK) and the Christie NHS Foundation Trust (UK) in Feb and Apr 2019 respectively. The double-strand break (DSB) yield of each modality was determined to compare the efficacy of electrons to that of gamma rays with respect to DNA damage.

INTRODUCTION AND AIMS

A recent study by Cancer Research UK indicates that one in two people born after 1960 will suffer from cancer during their lifetime [1]. Radiotherapy in the UK is primarily carried out using 12 MV photons, with 40% of patients receiving radiotherapy as part of their treatment [2] - typically in combination with surgery and/or chemotherapy.

Developments in high-gradient linear accelerators [3-5] could allow Very High Energy Electron (VHEE) therapy (involving the use of 50-250 MeV electrons), to become a viable option for radiotherapy treatment [6,7]. By adapting high-gradient technology, medical linacs with accelerating gradients of ~100 MeV/m could be capable of producing 250 MeV electrons within current treatment facilities.

VHEE therapy has several characteristics and potential advantages which make it an exciting area of radiotherapy research. Firstly, VHEEs have the potential to be used for treatment of deep-seated tumours – 200 MeV electrons can penetrate more than 30cm into tissue. Secondly, research at CERN's CLEAR facility indicates that VHEEs show relative insensitivity to inhomogeneities [8], making them suitable for treatment of heterogeneous regions, e.g. the lung.

As electrons are light compared to protons, they are more readily controllable using focusing and steering magnets, allowing rapid delivery. Sub-second treatment delivery could 'freeze' physiological motion, resulting in dose delivery with increased accuracy and reduced healthy tissue irradiation. In addition, these high dose rates (in excess of 40 Gy/s) appear to maintain tumour control while reducing radiation toxicity to healthy tissue [9-11].

The primary mechanism behind radiotherapy is DNA damage. Ionising radiation can cause several types of damage to DNA. The most difficult to repair are damages to one or both DNA strands – single-strand breaks (SSBs) and double-strand breaks (DSBs) respectively. If breaks are left unrepaired, or repaired incorrectly, the cell may be unable to function or replicate, potentially resulting in cell death.

Here, we investigate DNA damage caused by 6 - 15 MeV electron irradiation of plasmid DNA. Plasmids are ring-like DNA structures found in bacteria. Plasmids were chosen over cells as they have no repair mechanisms - pure DNA damage can be measured, as no DNA strand breaks will be repaired. This work is a prelude to cell irradiations – comparison of cell and plasmid irradiation will indicate the DNA damage repair (DDR) rate of irradiated cells.

The resulting DSB yields were then compared with those caused by similar irradiation using Co-60 gamma rays. This study contributes to a wider investigation into the biological effects of VHEE, with the aim of producing a value for the Relative Biological Effectiveness (RBE) of VHEE – RBE is the ratio of doses required by two radiation modalities to cause the same level of biological effect. It is normalised with respect to Co-60 γ RBE.

The following section outlines the setup of the plasmid irradiation experiments at the Dalton Cumbrian Facility (DCF) and the Christie. A section detailing the subsequent analysis techniques, mathematical models and method of calculating DSB yields will follow. The paper will conclude with a discussion of the experiment results and their fits to general models and an outline of future plasmid and cell irradiation studies.

EXPERIMENTAL SETUP

Plasmid Sample Preparation

pBR322 plasmid DNA, a commonly used cloning vector in *E. coli* with length 4361 base pairs (b.p.) was irradiated. It was purchased from New England Biolabs Inc, with initial concentration of 1000 ng/μl and diluted with double-distilled water to an assumed concentration of 100 ng/μl. Initial agarose gel electrophoresis of unirradiated sample indicated that >90% of the plasmid was in a supercoiled form. 15 μl (for Co-60 irradiation) or 30 μl (for electron irradiation) of the prepared plasmid solution, containing 1.5 or 3 μg of plasmid, was pipetted into 1.5 ml sealable Eppendorf tubes. Sample sizes were chosen based on a previous proton plasmid irradiation study by Vysin *et al.* [12].

Co-60 Gamma Irradiation – Dalton Cumbrian Facility

Gamma irradiation was carried out at the Dalton Cumbrian Facility (DCF). The irradiator was a Foss Therapy

Services FTS812 self-contained Co-60 γ irradiator, delivering gamma rays with energies 1.17 and 1.33 MeV.

Due to differences in dose rate depending on the position of samples in the chamber, the sample tubes were affixed to a turntable inside the chamber with rotation rate 12 rpm. This ensured a uniform total dose to each sample.

Doses of 5, 10, 20, 30, 40 and 50 Gy were delivered to four sets of six plasmid samples. Gafchromic EBT-XD film was also irradiated over a dose range of 5 – 25 Gy for dose calibration and cross-checking with the machine (not reported). A control sample was also prepared to provide plasmid proportions at zero dose.

The dose rate was measured using a RadCal 10X6-0.18 ionisation chamber. Due to the low doses required and the turntable speed, an initial average dose rate of 102.15 Gy/min was lowered to ensure an accurate, prolonged, uniform dose distribution. Three 12 mm thick lead blocks were used as attenuators and one of three Co-60 sources used, reducing the average dose rate to ~2.5 Gy/min.

Electron Irradiation at 6 – 15 MeV

Low-energy electron irradiation was carried out in Radiotherapy Suite 6 at the Christie NHS Foundation Trust. An Elekta Synergy unit was used, with the gantry at 90° to the treatment table. A filter was attached to provide a 20x20 cm² uniform flat field as shown in Fig. 1.

Eppendorf tubes holding 30μl of plasmid solution were slotted into a custom-designed sample holder and placed on the treatment table, 2cm from the beam window. The dose delivered was adjusted to account for this distance.

As with Co-60 irradiations, doses of 5 – 50 Gy were delivered to the samples and a control sample was included.
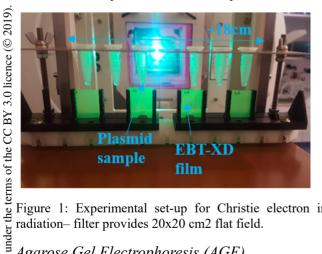

Figure 1: Experimental set-up for Christie electron irradiation– filter provides 20x20 cm2 flat field.

Agarose Gel Electrophoresis (AGE)

Agarose gel electrophoresis is a commonly used technique in molecular biology and biochemistry to separate and analyse a mixture of macromolecules. In this study, the resulting irradiated plasmid samples required separation into their different forms. Damage from ionising radiation causes the plasmid to adopt a different form.

Undamaged plasmid exists in a supercoiled (SC) form. When one strand is damaged (SSB), the plasmid relaxes into an open-circular (OC) form. When both strands are damaged (DSB), the plasmid adopts a linear (L) form [13].

The irradiated plasmid samples were split into 5 μl sub-samples. Each was mixed with 1 μl gel loading dye and pipetted into 5 mm wells in a 1% w/v agarose gel in 1x TAE buffer. The samples were run at 100 V in 0.5x TAE buffer for ~100 min or until the samples had migrated 70-80% through the gel.

The gels were imaged using a ChemiDoc MP UV imager (BioRad). Image analysis was carried out using ImageJ to determine the intensities of bands corresponding to the proportion of SC, OC and L plasmid forms - shown in Fig. 2.
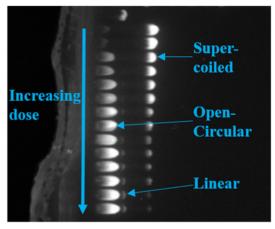

Figure 2: Gel image showing supercoiled (SC), open-circular (OC) and linear (L) bands with increasing dose.

MODELLING OF PLASMID DAMAGE

Two mathematical models were used to calculate the break yields from the supercoiled, open-circular and linear plasmid proportions. The first model was presented by Cowan et al. [14] and the second by McMahon and Currell [15].

The Cowan model was developed to predict DNA damage caused by enzymes 'nicking' random sites on the DNA strand. It consists of the following equations:

$$SC(D) = \frac{e^{-(\mu_0 + \mu D)}}{1 + \phi_0 + \phi D} \qquad (1)$$

$$OC(D) = \frac{1 - e^{-(\mu_0 + \mu D)}}{1 + \phi_0 + \phi D} \qquad (2)$$

$$L(D) = \frac{\phi_0 + \phi D}{1 + \phi_0 + \phi D} \qquad (3)$$

where μ and φ are the average yield of SSBs and DSBs per mega-base pair (Mbp) per Gray respectively, D is the delivered dose in Gray (Gy) and μ_0 and ϕ_0 are the initial μ and φ parameters at zero dose.

The McMahon model was developed to fit the results of AGE-measured plasmid damage resulting from irradiation.

$$SC(D) = S_0 e^{-(\mu D + \phi D)} \qquad (4)$$

$$OC(D) = e^{-\phi D} \left[e^{-\frac{1}{2}\mu^2 \rho D^2} (S_0 + C_0) - S_0 e^{-\mu D} \right] \quad (5)$$

$$L(D) = 1 - (S_0 + C_0) e^{-\left(\phi D + \frac{1}{2}\mu^2 \rho D^2\right)} \qquad (6)$$

where, as in the Cowan model, where μ and φ are the average yield of SSBs and DSBs per mega-base pair (Mbp) per Gray. S_0 and C_0 are the supercoiled and open-circular proportions at zero dose respectively and ρ is the probability of a DSB being formed from by two SSBs on opposite strands within 10 b.p. – for pBR322 plasmid, $\rho = 10/4361$ (where 4361 is the number of base pairs for pBR322). This is the primary physical difference between the McMahon and the Cowan model – the Cowan model does not contain the parameter ρ, hence DSBs formed by two nearby SSBs are not taken into account.

RESULTS

DCF Co-60 Gamma Irradiation

On analysis of the Co-60 γ images, the proportions of SC, OC and L plasmid were plotted against received dose. We made a least-square error non-linear fit for each model to the SC and OC data. The agreement in the L models (seen in (3) and (6)) with the linear proportion data indicates the efficacy of the models (Fig. 3).

Values of φ corresponding to the DSB yields for Co-60 γ irradiations were found to be 1.14 ± 0.05 and 1.41 ± 0.07 Mbp^{-1} Gy^{-1} using the Cowan and McMahon models respectively. These results were used as a baseline for comparison with electron break yields, as RBE is normalised with respect to Co-60 γ.
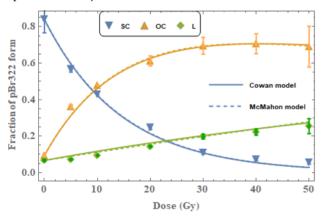

Figure 3: Data points for SC, OC and L proportions vs. dose for Co-60 γ irradiation and fits - solid and dashed lines representing Cowan and McMahon fits respectively.

Christie Electron Irradiation and Comparison

Plasmid form proportions were again plotted against dose and fitted to the Cowan and McMahon models after irradiation by 6 - 15 MeV electrons and AGE – 15 MeV data and fits shown in Fig. 4. SSB and DSB yields were calculated and are shown in Tables 1 and 2 respectively.

Following the break yield calculations for both modalities, the efficacy of electrons could be compared with that of Co-60 γ by directly comparing the DSB yields for both radiation modalities (electron DSBs found in Table 2). Electron DSB yields were found to increase with energy and Linear Energy Transfer (LET). 15 MeV electron and

Co-60 γ irradiations resulted in comparable DSB yields while 6 and 10 MeV were comparatively lower – as expected due to lower LET at these energies.

Table 1: SSB yields for 6 – 15 MeV Electron Plasmid Irradiation Calculated Using McMahon and Cowan Models

Energy (MeV)	LET (keV/μm)	McMahon μ (Mbp⁻¹ Gy⁻¹)	Cowan μ (Mbp⁻¹ Gy⁻¹)
6	0.1911	7.96±0.14	7.94±0.14
10	0.1968	11.00±0.65	11.00±0.66
15	0.2014	7.76±0.03	7.71±0.03

Table 2: DSB yields for 6 – 15 MeV Electron Plasmid Iirradiation Calculated Using McMahon and Cowan Models

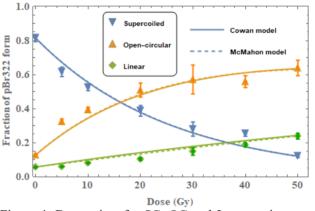| Energy (MeV)	LET (keV/μm)	McMahon φ (Mbp⁻¹ Gy⁻¹)	Cowan φ (Mbp⁻¹ Gy⁻¹)
6	0.1911	0.81±0.08	0.97±0.10
10	0.1968	0.88±0.07	1.13±0.08
15	0.2014	1.00±0.01	1.22±0.01

Figure 4: Data points for SC, OC and L proportions vs. dose for 15 MeV e- irradiation and fits - solid and dashed lines representing Cowan and McMahon fits respectively.

CONCLUSIONS

Plasmid irradiation experiments were carried out using 6 – 15 MeV electrons at the Christie and Co-60 γ at DCF. DSBs are caused by electrons, with yield increasing with energy and LET. DSB yields for 15 MeV electrons and Co-60 γ were similar, indicating that electrons could be as effective at damaging DNA as Co-60 γ.

Further plasmid and cell irradiations are planned at higher energies using the CLARA (Daresbury Laboratory) and CLEAR (CERN) facilities to allow calculations of electron RBE based on DNA damage and cell survival endpoints. Such work may result in an improved understanding of the efficacy of VHEE as a radiotherapy treatment.

ACKNOWLEDGEMENTS

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