Applied Radiation and Isotopes 128 (2017) 114–119

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

journal homepage: www.elsevier.com/locate/apradiso

In vitro investigation of the dose-rate effect on the biological effectiveness of megavoltage X-ray radiation doses

Sianne Oktaria[a,b,c], Michael L.F. Lerch[a], Anatoly B. Rosenfeld[a], Moeava Tehei[a,b,c], Stéphanie Corde[a,d,*]

[a] Centre for Medical Radiation Physics, University of Wollongong, Wollongong, NSW 2522, Australia
[b] Centre for Medical and Molecular Bioscience, University of Wollongong, Wollongong, NSW 2522, Australia
[c] Illawarra Health and Medical Research Institute, Wollongong, NSW 2522, Australia
[d] Radiation Oncology Department, Prince of Wales hospital, Randwick, NSW 2031, Australia

HIGHLIGHTS

- We examined the biological effect of differing dose-rates for 10 MV from a LINAC.
- Cell survival curves were used to determine the α and β values (radiosensitivity).
- A reduction in dose rate has no effect on the survival curve of 9L cells.
- A lower dose rate killed more MCF-7 cells.
- We showed that dose-rate is important in determining the efficacy of IMRT.

ARTICLE INFO

Keywords:
Inverse dose-rate
Megavoltage
Radiation therapy

ABSTRACT

Radiation therapy is rapidly evolving toward the delivery of higher dose rates to improve cancer treatment. *In vitro* experiments were performed to investigate the response of 9L and MCF-7 cancer cell lines, exposed to 10 MV X-ray radiations. Up to 8 Gy was delivered at a dose-rate of 50 cGy/min compared to 5 Gy/min. The data obtained emphasizes the importance of taking into account not only the physical, but also the radiobiological parameters, when planning a particular cancer treatment.

1. Introduction

Radiation therapy (RT) is an important modality for many cancer treatments, with over 50% of cancer patients receiving some form of RT as part of their cancer management plan. The ongoing challenge in RT treatment is the controlled delivery of a lethal dose to the tumor whilst minimizing damage to the surrounding normal tissue. Modern radiotherapy techniques such as intensity modulation radiation therapy (IMRT), intensity modulated arc therapy (IMAT) or volumetric-modulated arc therapy (VMAT), and stereotactic radiotherapy (SRT) (Benedict et al., 2001; Siochi, 1999; Tubiana and Eschwege, 2000) have improved local tumor control through better precision of the radiation dose delivered. These technologies consequently often deliver more complex treatment fields than conventional techniques.

Clinically, IMRT has become an important modality and it has been widely used in radiotherapy for over 15 years. The capabilities of IMRT have been extensively described in the literature in physical term advantages, such as target coverage conformity, better dose uniformity, and sparing the adjacent normal tissue. These make IMRT superior to conventional or three-dimensional conformal external radiotherapy (3D-CRT) (Cheung, 2006; Intensity Modulated Radiation Therapy Collaborative Working Group, 2001). However, in IMRT, an increased number of monitor units (MU's) are required, and thus IMRT (beam-on time) generally involves a longer dose delivery time than conventional RT. The radiobiological advantages of IMRT have been extensively debated in the literature (Fowler et al., 2004; Ling et al., 2010; Lohse et al., 2011; Moiseenko et al., 2007; Mu et al., 2003; Sorensen et al., 2011; Wang et al., 2003). The suspicion that IMRT could decrease tumor control due to the increase in the overall treatment time does not have any clinical evidence, and is offset by the advantages linked to the better conformation of physical doses to the target volumes and therefore better sparing of critical organs.

Improvement in clinical outcomes can be achieved by reducing patient intra fraction movements. Recent technical developments have

* Correspondence to: Radiation Oncology Department, Prince of Wales Hospital, High St, Randwick, NSW 2031, Australia.
E-mail address: scorde@uow.edu.au (S. Corde).

http://dx.doi.org/10.1016/j.apradiso.2017.07.008
Received 14 November 2016; Received in revised form 24 May 2017; Accepted 5 July 2017
Available online 06 July 2017
0969-8043/

therefore focused on increasing the clinical dose rates to minimize the effect of such movements. Increasing the dose rate also has the added benefit of reducing the overall patient treatment time thus allowing an increase in patient throughput. One recent development is the removal of flattening filters in the linear accelerator heads (flattening filter free (FFF) LINAC configuration), which has proven to be particularly beneficial for IMRT and SRT (Fu et al., 2004; Kragl et al., 2009; Stathakis et al., 2009). The absence of the flattening filter, leading to a significant decrease in the number of MU's for a given photon treatment delivery, has been reported in the literature for both Varian (energies 6 MV and 18 MV) (Stathakis et al., 2009) and Elekta (energies 6 MV and 10 MV) (Kragl et al., 2009) clinical linear accelerators. It is thus important to, and would be hazardous not to, investigate the biological effectiveness of physical radiation doses delivered with differing dose rates.

Some recent data published related to this has emphasized the radiobiological effect of a high instantaneous dose rate and indicated that there is no effect of the instantaneous dose rate of FFF LINACs on clonogenic cell survival (Sorensen et al., 2011). Other data however e.g. Lohse et al. (2011), shows that the radiobiological effect of the FFF beam is dependent on the dose per pulse and suggests that this might become a crucial factor that influences cancer cell survival. Ling et al. (2010) reviewed the dose rate effect in external beam radiotherapy and concluded that it is the overall beam-on time that determines the tumor cell survival, not the average dose-rate of the linear accelerator (LINAC) nor the instantaneous dose-rates within LINAC pulses. In this work, we are primarily focused on the overall time of irradiation and its influence on the survival of two cell lines with significantly different radiosensitivities.

Dose-rate sparing is usually known to involve a decreased biological response to radiation exposure at a low dose rate compared to a high dose rate, as theoretically predicted by Lajtha and Oliver (1961). On the contrary, Mitchell *et al.* first identified the decrease in survival of HeLa cells irradiated at a low dose rate of 37 cGy/h compared with a high dose rate of 1.54 Gy/h and this was referred to as the "inverse dose-rate effect" (Mitchell and Bedford 1977; Mitchell et al., 1979b). Similar studies by Furre et al. (1999), also observed an inverse dose-rate effect on NHIK 3025 cells. These data suggest that for some cell lines, a monotonic increase in dose-rate does not produce a similar increase in cell killing. Moreover, in some specific cell lines, increasing the dose rate actually decreases the cell killing effectiveness.

In this article we report on results of *in vitro* experiments in which the biological effect of differing dose-rates for 10 MV X-ray irradiations delivery from a LINAC has been assessed by colony forming assay. The dose is delivered using clinical dose rates of 50 cGy/min compared to a 10-fold higher dose rate of 5 Gy/min. 9L and MCF-7 cell lines were used because they represent a good pre-clinical model of brain and breast tumor tissues respectively. Their intrinsic radiosensitivity is different, with 9L being considered to be more radiation resistant than the MCF-7. To our knowledge, this is the first time that the biological effectiveness of 10 MV X-ray radiation doses on 9L and MCF-7 cell lines has been investigated. The significance in the findings of this study are discussed with regard to observations on the radiosensitivity of the 9L and MCF-7 cells, concepts of radiobiology, and potential implications for new methods of dose delivery (IMRT, IMAT, VMAT, etc.).

2. Material and methods

2.1. Cell lines

9L, an adherent, fibroblast-like, radio-resistant rat gliosarcoma cell line derived from N-nitrosomethylurea-induced tumor was obtained from the European Collection of Cell Cultures (ECACC). MCF-7 is an adherent, epithelial-like, radiosensitive breast adenocarcinoma cell line established from a 69-year-old female (American Type Culture Collection – ATCC). Both cell lines were tested routinely for mycoplasma contamination. Cells were maintained in exponential growth in

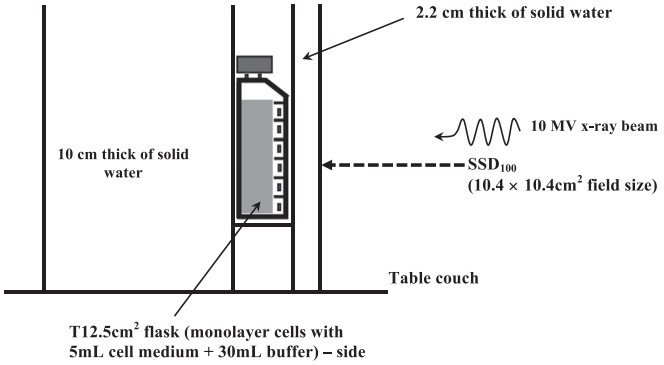

Fig. 1. Experimental setup for cellular irradiation.

DMEM (Invitrogen, AU) with L-Glutamine and supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Pen Strep) at 37 °C in a humidified incubator with 5% CO_2 in air.

2.2. Irradiation procedures

The irradiations were performed at the radiation oncology department in the Prince of Wales hospital, Randwick, Australia, using an Axesse Elekta LINAC with beam modulator (Elekta AB, Kungstensgatan, Stockholm, Sweden) operated at energy of 10 MV. Single exposure with doses of 1, 2, 3, 5, and 8 Gy were delivered at dose rates of 50 cGy/min and 5 Gy/min.

Experiments were carried out with confluent cultures grown as monolayer in 12.5 cm^2 cell culture flasks with a vented screw cap (BD Falcon) containing 5 mL of DMEM medium and 30 mL of Hanks' balanced salt solution (HBSS). The cells flasks were placed vertically facing the beam and were positioned at a depth of 2.2 cm in solid water to match the D_{max} depth of the 10 MV photon field. An additional thickness of 10 cm of solid water was placed behind the flask to assure adequate scattering conditions. To maintain electronic equilibrium conditions within the flasks during the irradiation, the flasks were also surrounded by solid water slabs. The irradiation field size used for all experiments was 10.4 × 10.4 cm^2 and the source-to-surface distance (SSD) was 100 cm. Fig. 1 shows a schematic diagram of the setup for cellular irradiation. Cell culture flasks were irradiated at room temperature. Unirradiated control samples were kept at room temperature in the control room (i.e at the same condition as the irradiated samples – full of HBSS buffer and placed vertically) while the other samples were irradiated in the LINAC bunker.

2.3. Clonogenic survival

Cell survival was measured by the colony-forming assay, i.e. the ability of a single cell to form colonies *in vitro* (Puck and Marcus, 1956). The method used in this study was derived from previous work (Oktaria et al., 2015). Both the control and irradiated cells were plated immediately after the irradiation experiments. The medium was removed and the confluent cells were washed gently with DPBS (Ca^{2+} and Mg^{2+} free), then detached using Trypsin-EDTA. The disassociated cells were counted with a haemocytometer and seeded at low densities to achieve approximately 100 colonies after fifteen doubling times into 100-mm tissue culture dishes (BD Falcon) containing 10 mL of complete cell medium (i.e. DMEM containing L-Glutamine and supplemented with 10% (v/v) FBS and 1% (v/v) PenStrep). Depending on the prescribed dose, each experiment involved a maximum of three cells densities with triplicate dishes for each density. The numbers of cells plated per petri dish were determined by preliminary experiments designed in order to determine the radiosensitivity of the cell lines. The cells in petri dishes were then incubated to allow colonies to form at 37 °C humidified 5% (v/v) CO_2 cell culture incubator (HERACELL 150i). After fifteen