Physica Medica 31 (2015) 615–620

Contents lists available at ScienceDirect

Physica Medica

journal homepage: http://www.physicamedica.com

Original paper

Improved normal tissue protection by proton and X-ray microchannels compared to homogeneous field irradiation

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ARTICLE INFO

Article history:
Received 20 December 2014
Received in revised form
2 April 2015
Accepted 10 April 2015
Available online 27 April 2015

Keywords:
Microbeams
Protons
X-rays
In vitro skin model

ABSTRACT

The risk of developing normal tissue injuries often limits the radiation dose that can be applied to the tumour in radiation therapy. Microbeam Radiation Therapy (MRT), a spatially fractionated photon radiotherapy is currently tested at the European Synchrotron Radiation Facility (ESRF) to improve normal tissue protection. MRT utilizes an array of microscopically thin and nearly parallel X-ray beams that are generated by a synchrotron. At the ion microprobe SNAKE in Munich focused proton microbeams ("proton microchannels") are studied to improve normal tissue protection. Here, we comparatively investigate microbeam/microchannel irradiations with sub-millimetre X-ray versus proton beams to minimize the risk of normal tissue damage in a human skin model, *in vitro*. Skin tissues were irradiated with a mean dose of 2 Gy over the irradiated area either with parallel synchrotron-generated X-ray beams at the ESRF or with 20 MeV protons at SNAKE using four different irradiation modes: homogeneous field, parallel lines and microchannel applications using two different channel sizes. Normal tissue viability as determined in an MTT test was significantly higher after proton or X-ray microchannel irradiation compared to a homogeneous field irradiation. In line with these findings genetic damage, as determined by the measurement of micronuclei in keratinocytes, was significantly reduced after proton or X-ray microchannel compared to a homogeneous field irradiation. Our data show that skin irradiation using either X-ray or proton microchannels maintain a higher cell viability and DNA integrity compared to a homogeneous irradiation, and thus might improve normal tissue protection after radiation therapy.

Introduction

In radiotherapy, the risk of developing normal tissue injuries often limits the radiation dose that can be applied in tumour patients. Radiation damage in the skin can reduce the patient's quality of life after tumour therapy. Microbeam Radiation Therapy (MRT), a spatially fractionated radiotherapy, uses an array of microscopically thin and nearly parallel synchrotron-generated X-ray beams [1–3]. In X-ray MRT the tumour is exposed to arrays of narrow 25–75 μm wide microplanar beams. These parallel orientated beams are separated by distances of typically 50–400 μm. The microarray geometry is maintained in the tumour, and comparisons between broad beam irradiations and MRT indicate a higher therapeutic index due to less toxicity in the normal tissue and a selective radiovulnerability of the tumour vasculature versus normal blood vessels by MRT [4–6].

Similar approaches use focused proton microbeams, here termed "microchannels", which remain separated in normal tissues but spread out until they reach the tumour to achieve a homogeneous dose distribution inside the target volume. The rationale for proton microchannels is therefore the reduction of normal tissue toxicity (as in X-ray MRT), while the response of the target volume is unaffected. The microchannel approach has been established at the ion microprobe SNAKE in Munich [7]. A microchannel

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http://dx.doi.org/10.1016/j.ejmp.2015.04.004

irradiation at SNAKE with 20 MeV protons resulted in reduced inflammatory response in a human skin model compared to conventional homogeneous broad-beam irradiation [7]. They applied protons either focused in a matrix of 500 × 500 μm^2 (total field size: 4 × 4 mm^2) using 50 × 50 μm^2 wide channels with a channel dose of 200 Gy, or homogeneously with the same mean dose of 2 Gy. Inflammation was determined by measuring soluble inflammatory response parameters such as Interleukin-6, TGF-beta and Pro-MMP1 in the supernatant of the human skin tissue. The results showed a lower inflammatory response in the human skin model after microchannel compared to homogeneous irradiation. No significant cell death was observed in the skin after irradiation with 50 μm microchannels compared to non-irradiated controls, while homogeneously irradiated tissues showed a decrease in the cell viability of 48%.

In 2013, another group published Monte Carlo simulations for microchannel proton irradiations [8]. The authors evaluated peak-to-valley doses for several arrays of proton minibeams and concluded possible tissue sparing effects due to the spatial fractionation of the dose.

The major goal of the present study was to investigate if microchannel irradiation (using a similar concept as “pencilbeam” or “minibeam” irradiations, cf. [9] and [8]) in the micrometre size range of X-ray versus proton beams can also minimize the risk of normal tissue damage. Therefore, viability and genetic damage was determined in a three-dimensional human skin model after four different microbeam irradiation modes: a homogeneous field (HF), lines (LN), and small and large channels (SC and LC) with synchrotron-generated X-ray beams at the ESRF (Grenoble, France) and proton irradiation at the SNAKE microbeam (Munich, Germany).

Materials and methods

Tissue construct

The three-dimensional full-thickness skin model EpiDermFT™, which was used in previous studies [7], was obtained from MatTek Corporation, Ashland, MA, USA. For the irradiations at SNAKE the EFT-400 with a surface area of 1 cm^2 (thickness roughly half a millimetre) and for the irradiations at the ESRF the EFT-300 was used, which is the same as EFT-400 except the surface area was only 0.9 cm^2. This reconstructed human skin is a differentiated tissue consisting of cornified, granular, spinous and basal layer like the normal human epidermis, and a dermal layer. EpiDermFT™ is mitotically and metabolically active. The tissue, consisting of human-derived epidermal keratinocytes and dermal fibroblasts, is cultured on special cell culture inserts in 12-well plates, each containing 2.0 ml of fresh 37 °C New Maintenance Medium (NMM, MatTek Corporation, Ashland, MA, USA). The samples were incubated at 37 °C in a humidified atmosphere (5% CO_2), replacing the culture medium every 24 h.

Irradiation conditions at SNAKE (protons)

Proton irradiation was carried out at the Munich ion microprobe SNAKE (Superconducting Nanoprobe for Applied nuclear [Kern] physics Experiments) of the 14 MV Munich tandem accelerator, where defined cell nuclei can be irradiated with single or counted protons, thus allowing a precise dose application [10–12]. During irradiation, the skin construct was retained in a specially designed container (cf. [7,12]) and mounted directly behind the beam exit nozzle, with the dermis facing the beam. For an exact dose calculation, every proton was detected in a scintillator-photomultiplier detector after traversing the skin sample [7], with an LET value of 2.66 keV/μm at the position of the skin sample. The same irradiation setup was used to prepare the beam for two focused modes (channel modes) and for a homogeneous mode. In all three cases the average dose over the irradiated area was 2 Gy, with an uncertainty of approximately 4%, estimated mainly from the uncertainty of the field size (~2% in each dimension) and the accuracy of the LET value (~1–2%) [13,7]. For the microchannel irradiations, the channel size and distance was chosen such that 1% of the skin was irradiated. Small channels (SC, 50 × 50 μm^2, as in Ref. [7]) and large channels (LC, 180 × 180 μm^2) were irradiated with a local dose of 200 Gy and the distances between the channels were 500 μm (centre-to-centre) and 1800 μm, respectively. Three skin samples were used for each irradiation mode, and the whole experiment was performed in duplicate.

Irradiation conditions at ESRF (X-rays)

A very similar irradiation was performed with X-rays at the ID17 beamline of the European Synchrotron Radiation Facility (ESRF) in Grenoble/France, which is also used for the MRT activities at ESRF. It is equipped with a multi-slit tungsten collimator and a goniometer which can move the sample through the beam in order to generate microplanar beams as used in MRT [3]. The energy spectrum ranges from 50 to 350 keV with the maximum at around 100 keV. A specially designed tissue holder allowed the skin sample to be mounted on the goniometer, with the dermis facing the source. The skin sample was sandwiched between 2 mm of PMMA towards the source (to provide sufficient build-up) and 9 mm water (medium) followed by 6 mm of PMMA on the other side. A circular tungsten mask with 4.6 mm diameter was mounted immediately upstream of the sample.

For this setup, four irradiation modes were prepared: a homogeneous field (HF), lines (LN), and small and large channels (SC and LC). The vertical lines had a thickness of 51 μm and a distance (centre-to-centre) of 412 μm (defined by the multi-slit collimator), with the sample moving through the beam as for MRT. By using one horizontal line of 51 μm width with the same multi-slit collimator settings, a linear array of small channels of 51 × 51 μm^2 with a distance (centre-to-centre) of 412 μm could be generated. Vertical stepping of the sample with a step size (centre-to-centre) of 607 μm produced the desired SC pattern with one channel per 0.25 mm^2, very similar to the SC pattern realized at SNAKE. A large channel (LC) with 180 × 180 μm^2 was generated by primary slits (without the multi-slit collimator, and without the 4.6 mm collimator), and the sample was moved horizontally and vertically in steps of 1800 μm (centre-to-centre) in order to realize the same pattern as for large channels at SNAKE. Three skin samples were used for each irradiation mode.

In all four irradiation modes, the intended average dose over the irradiated area was 2 Gy. The required peak doses and output factors for irradiation (relative to a pin point ionization chamber measurement in an open field of 10 × 10 mm^2) were determined by detailed Monte Carlo simulations in Geant4 version 9.3p2 on a lateral calculation grid with a resolution of 5 μm. The Livermore low energy physics libraries were employed with 1 μm cut of lengths. These simulations were used to determine the mean dose over the nominal field size and to scale this dose to 2 Gy for all irradiation modes. Due to the shallower beam penumbra compared to protons, the resulting peak doses were lower than for protons (e.g. 186 Gy for SC and 15.1 Gy for LN compared to 200 Gy at SNAKE), with valley doses for X-rays of around 0.03 Gy.

MTT tissue viability test

The cell viability of the complete skin (i.e. keratinocytes in the epidermis and fibroblasts in the dermis) was quantified 40 h after