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Currently used in clinical practice beam rate changes have no significant effect on the reduction of clonogenic capacity of PNT1A cells in vitro

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ABSTRACT

Background: Due to the lack of selectivity of ionizing radiation between normal and cancer cells, it is important to improve the existing radiation patterns. Lowering the risk of cancer recurrence and comfort during treatment are priorities in radiotherapy.

Materials and methods: In the experiment we used dose verification to determine the irradiation time calculated by a treatment planning system for 6XFFF and 10XFFF beams. Cells cultured under standard conditions were irradiated with a dose of 2 Gy at different beam rates 400 MU/min, 600 MU/min, 800 MU/min, 1000 MU/min, 1400 MU/min, 1600 MU/min and 2400 MU/min using 6XFFF, 10XFFF and 6XFF beams.

Results: The experiment was aimed at comparing the biological response of normal prostate cells after clinically applied radiation patterns. No statistically significant differences in the cellular response were observed. The wide range of beam rates as well as the beam profiles did not significantly affect cell proliferation.

Conclusions: High beam rates, without significantly affecting the clonogenic capacity of cells, have an impact on the quality of patient's treatment. With the increasing beam rate the irradiation time is shortened, which has an important impact on patients' health. This experiment can have a practical significance.

Key words: dose-rate; profile beam; radiotherapy; radiobiology; prostate

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Introduction

Cancer is one of the leading causes of mortality in developed societies while being also a significant global public health problem. The critical aspect in this matter is the development of conventional therapeutic methods [1, 2]. Many different factors contribute to successful treatment, e.g., the choice of the type of therapy, the appropriate optimization of therapy, duration of treatment, quality of life, prevention of further progression and disease, treatment efficiency, side effects and costs [3]. One of the main methods of cancer treatment is radiotherapy in addition to surgery, chemotherapy, immunotherapy, and hormone therapy [4]. It is estimated that in Europe, about half a million people a year are treated with this type of treatment [5]. Radiotherapy uses megavolt radiation to damage

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the genetic material of tumour cells. Ionising radiation affects both cancer cells and healthy cells, but ultimately the percentage of eradicated cancer cells should be maximised using the differences in the radiobiological response of these two types of tissues [6, 7]. Healthy tissues, depending on the structure and function, have a different tolerance to ionising radiation. Recent radiobiological studies allow a better understanding of pathogenesis processes leading to radiation toxicity. Generally, the level of structural damage depends on the characteristic properties of the tissues' radiosensitivity [8]. Searching for the underlying mechanisms for radiation toxicity requires careful monitoring of a number of factors. We can determine their impact based on observed response to various applied treatment schemes [9]. There are many different technical factors in therapeutic radiation treatment that have a varying effect on the course and impact of therapy. These factors have to be considered during treatment planning and dosimetry verification. The most significant of them include beam energy, beam profile, dose, and dose or beam rate (DR). In this work, we wanted to analyse differences in the radiobiological response of healthy prostate cells using unflattened (FFF) and flattened (FF) beams with different energy and beam rate. Flattening filter free beams increase the beam rate compared to standard flattened beams, and this allows the duration of treatment to be reduced [10, 11]. The FFF beams also reduce leakage from treatment head [10, 12], neutron contamination for high energy beams [13], which results in lower out-of-field dose to healthy organs [14].

We decided to focus on the effect of beam rate, because the potential changes in beam rate may have a decisive influence on the effectiveness of radiotherapy as well as on the severity of both systemic and in-field healthy tissue side effects [15].

The main aim of this research was to investigate the biological response of normal prostate cells (PNT1A) exposed to ionising radiation delivered at different beam rates using 6XFFF, 10XFFF and 6XFF beams.

Materials and methods

Cell culture

In the experiment, the normal prostate cell line (PNT1A) was used. Cells were cultured in RPMI Medium 1640 (Gibco Invitrogen, Carlsbad, CA) supplemented with 10% of fetal bovine serum and 1% of penicillin/streptomycin (Gibco Invitrogen, Carlsbad, CA), and incubated at 37°C in an atmosphere enriched with 5% CO_2 with 95% humidity. Cells with min. 90% of confluence were passaged on average every 2/3 days. After reaching the appropriate confluence, the cells were plated on T-25 bottles.

Irradiation conditions

The cells culture bottles were placed in a central beam axis in the MP1 water Phantom (PTW, Freiburg, Germany), irradiated with 6XFF, 6XFFF and 10XFFF beams. The geometry of the measuring system is shown in Figure 1A. Cells were located during irradiation with the 6XFF antavd 6XFF beam at the depth of 5 cm, and during the irradiation with 10XFFF at 10 cm. For the SSD = 100 cm, the area of the irradiated field was 30x30 cm at isocenter to cover, with a homogenous dose, the entire surface of the bottle with cells. Photon beams were delivered with a TrueBeam (Varian Medical Systems, Palo Alto, CA, USA).

Dose verification

To verify the irradiation time calculated by treatment planning system for 6XFFF and 10XFFF beams, as well as to show the lack of dependence of the delivered dose on the beam rate, measurements were made using the ionisation chamber in the conditions identical with the experimental conditions (Fig. 1BC). The Semiflex chamber (PTW-Freiburg, Germany) type 31010 was used.

Clonogenic assay

After irradiation cells were plated on 6-well dishes in the number of 5×10^3 cells per well. After 14 days of culture, a single colony had 50 clones. Colonies were fixed with ethanol and stained with Coomassie blue, and counted. Plating efficiency (PE) was calculated to control, survival fraction (SF) was also determined based on previous calculations. The experiment was performed in triplicate.

Statistical analyses

Statistical analysis of the results was carried out with the Statistica V12.5 (purchased from: StatSoft Polska Sp. z o. o., Kraków, Poland). To analyse the

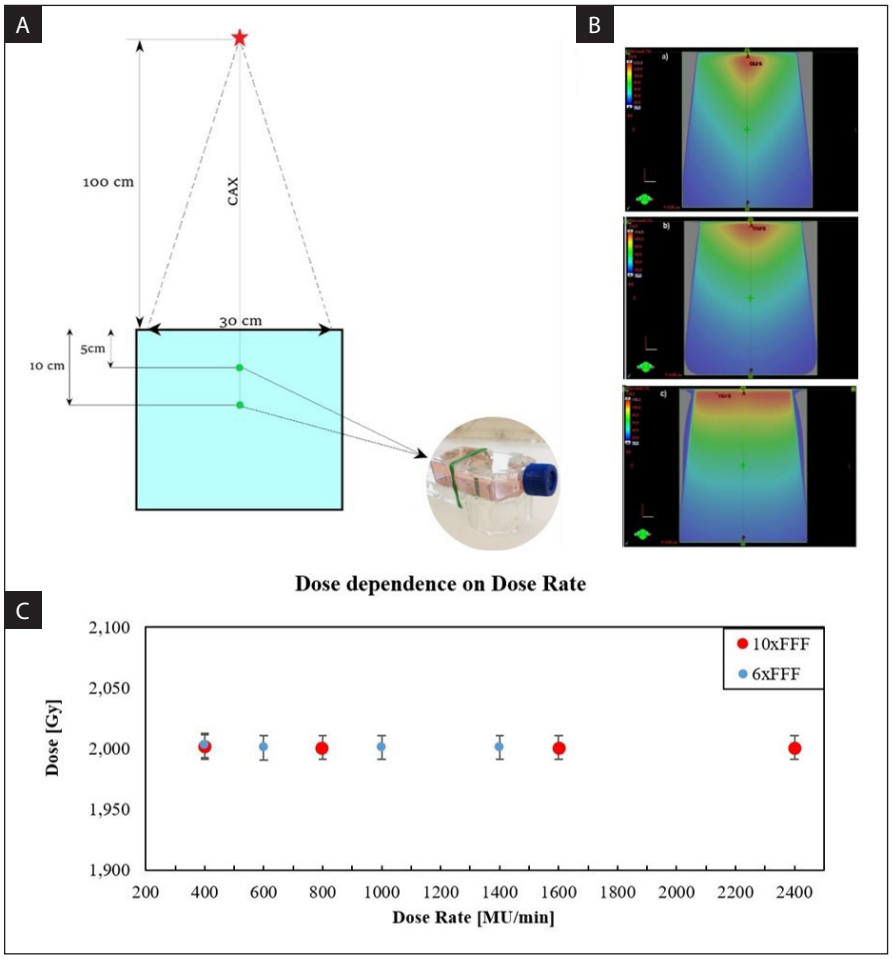

Figure 1. A. Geometry of the measuring system: PTW MP1 water phantom, irradiation field 30 × 30 cm for SSD = 100 cm, cells at the depth 5 cm (for 6XFF, 6XFFF) and 10 cm (for 10XFFF) in the central beam axis; **B.** Colour wash dose distribution for 10XFFF (a), 6XFFF (b) and 6XFF (c) beam using 30 × 30 cm irradiation field, calculated by the Eclipse treatment planning system: A — anterior; P — posterior; R — right; L — left; **C:** Graph shows the relation between the measured dose and beam rate of radiation (400 MU/min, 600 MU/min, 1000 MU/min, 1400 MU/min) for 6XFFF beam and for 10XFFF beam ((400 MU/min, 800 MU/min, 1600 MU/min, 2400 MU/min). The values presented in the graph are the mean of the obtained results and the deviation is the standard deviation. The experiment was performed in triplicate

normal distribution of the studied groups, the Shapiro-Wilk test was used. The analysis of the relationship between the two groups was made using the Student's t-test. The results of p level ≤ 0.05 was assumed as statistically significant.

Results

The experiment was conducted to compare the chosen prostate irradiation patterns. The primary purpose was to analyse the established radiation patterns, comparing the radiobiological response for the combinations of different beam rates and energies, with a fixed radiation dose of 2 Gy. We studied different beam rates for beam energy 6XFFF (Fig. 2A); different beam rates for beam energy 10XFFF (Fig. 2B); different beam rates for 6XFFF and 10XFFF beams (Fig. 2C); different beam rates for 6XFF and 6XFFF beam (Fig. 2D). Taking into account all the combinations, there are no noticeable differences ($p > 0.05$) between the results obtained for the radiobiological response of the normal prostate cells. In each of the graphs presented below, we observed no statistically significant changes in survival of cells irradiated with different radiation schemes. Figure 1 shows the dose distribution calculated by the Eclipse 13.6 treatment planning system (Varian Medical Systems, Palo Alto, CA, USA), which was used to assign the irradiation time. Table 1 presents obtained parameters that were used to provide 2 Gy dose to the cells at specific depths

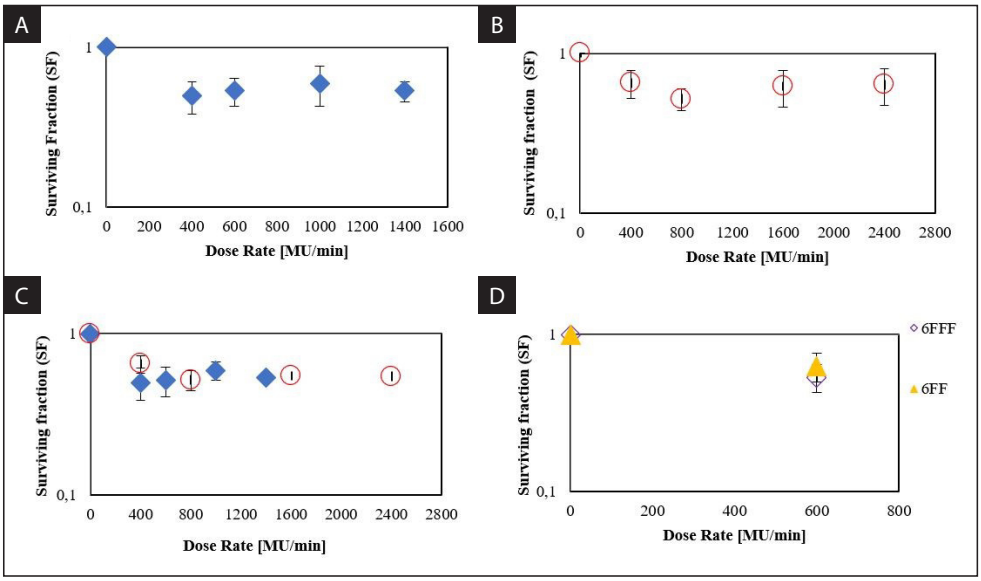

Figure 2. A. The graph shows the relation between the survival rate of the normal PNT1A prostate cell line and beam rate of radiation (400 MU/min, 600 MU/min, 1000 MU/min, 1400 MU/min). The radiation dose for individual beam rates was 2 Gy, delivered with 6XFFF beam. The results are means of three biological replicates; the error bars represent standard deviations; **B.** The graph shows the relation between the survival rate of the normal PNT1A prostate cell line and beam rate of radiation (400 MU/min, 800 MU/min, 1600 MU/min, 2400 MU/min). The radiation dose for individual beam rates was 2 Gy, delivered with 10XFFF beam. The results are means of three biological replicates; the error bars represent standard deviations; **C.** The graph shows the comparison of the survival of the normal PNT1A prostate cell line irradiated with different radiation schemes: Radiation scheme 1. Dose: 2 Gy, beam rate: 400 MU/min, 800 MU/min, 1600 MU/min, 2400 MU/min, energy: 6XFFF (blue diamonds). Radiation scheme 2. dose: 2 Gy beam rate: 400 MU/min, 800 MU/min, 1600 MU/min, 2400 MU/min, energy: 10XFFF (red circles); **D.** The graph shows the comparison of the survival of the normal PNT1A prostate cell line irradiated with different radiation schemes: 1. dose: 2 Gy, beam rate: 600 MU/min, energy: 6XFFF (violet diamond), 2. dose: 2 Gy dose beam: 600 MU/min, energy: 6XFF (yellow triangle)

Table 1. Experimental parameters: energy, beam rate, depth, time

6XFFF			10XFFF			6XFF		
Dose Rate [MU/min]	Depth [cm]	Time [MU]	Dose Rate [MU/min]	Depth [cm]	Time [MU]	Dose Rate [MU/min]	Depth [cm]	Time [MU]
400, 600, 1000, 1400	5	233	400, 800, 1600, 2400	10	234	600	5	182

Discussion and Conclusion

Ionising radiation causes a wide variety of DNA and protein damage. Cellular macromolecules provide signals activating protein sensors that stimulate cell cycle arrest and repair processes in response to radiation [16]. Both normal and cancerous cells respond differently to irradiation with a high or low dose rate [12]. The spectrum of clinically dose rates generated by linear accelerators (between 1 and 10 Gy/min) are too small for significant changes in the cellular response to emerging [17]. Ionising radiation can affect many processes taking place in the cell and, therefore, the effect of radiation depends, to a large extent, on its activity. The state of activity of a cell is determined by the activity of its genes, the propensity to mutations, and the microenvironment, including growth factors, cytokines, nutrients surrounding the cells, and the extracellular substance to which cells adhere. Radiotherapy can initiate processes occurring within the nucleus, cell membrane and cytoplasm, although DNA is considered the most important biological shield. It is challenging to apply external irradiation with meagre dose rate due to the discomfort of the patient subjected to a few hours of irradiation session necessary to deliver a given fractional dose. The results of clinical trials [18]

have shown that there is a large individual genetic background, varied in terms of cells radiosensitivity. Patients treated with the same dose of radiation according to the same fractionation scheme differ in the severity of early and late irradiation reactions, and show different tumor responses to radiation therapy. The finding of high sensitivity of normal cells may indicate patients' hypersensitivity and intensification of late radiation readings, and suggest a reduction in the total dose per tumor. We investigated the effect of different beam rates on normal prostate cells. Our results show that there is no statistically significant difference in the normal prostate cell surviving fraction after irradiation with chosen beam rates (between 400 and 1400 MU/min for 6XFFF beam, and between 400 and 2400 MU/min for 10XFFF beam). It leads to the conclusion that the substantial beam rate changes currently used in treatment have no significant effect on the reduction of clonogenic capacity of cells. The sequence of events occurring in the irradiated cell is not well understood. The cell receives signals from the outside through receptor systems found in the cell membrane, cytoplasm and nucleus. The inability to conduct signals is believed to be the cause of cell death. It may be due to damage to the receptors for signals or their conduction pathways, to missing or incorrect transcriptional function. The cause of this impairment may be damage to the genome and disruption of signalling pathways or the opening of others, such as apoptosis. A critical signal is DNA damage. Different types of damage can be distinguished at the molecular level in the DNA of irradiated cells. These include single strand breaks, double strand breaks, DNA protein crosslinks. Oktaria et al. [19] conducted in vitro experiments to investigate the response of breast cancer cell lines (MCF-7) and malignant glioma (9L), exposed to ionising radiation delivered with 10XFFF beam. For analysis, the survival curves were plotted to determine the effects of dose rates, to evaluate the radiosensitivity. Doses up to 8 Gy were used with selected DR values: 50 cGy/min and 5 Gy/min. Dose rate reduction did not affect the survival curve of 9L cells, whereas a decrease in proliferation was seen with MCF-7 with a decrease in DR. The obtained data emphasise the importance of considering not only physical but also radiobiological parameters when planning specific cancer treatment. Another group[20] used the linear accelerator Varian Trilogy TX for irradiation and compared the effects of three different dose rates (5.01, 9.99 and 29.91 Gy/min, with the current dose in impulse 56.5; 112.0, 8.0 and 338.0 Gy/s) on clonogenic survival. Normal lung (V79) and squamous cell carcinoma (FaDu) cells were irradiated with doses ranging from 1 to 10 Gy to obtain survival curves in response to dose. For both cell lines, no differences in cell survival were observed for cells treated with different DRs. Recently, a significant development in the field of technique and clinical approach in radiotherapy has been found. It has led to a re-analysis of the potential impact of dose rate as well as beam rate in clinically applied treatment plans. Ling et al. [21] estimated the effect of the overall treatment time in certain radiation schemes. Irradiation affects both healthy and malignant tissues. They noticed that late reacting healthy tissues are more susceptible to changes in dose rate than tumour cells or early reacting tissues. Radiotherapy is rapidly evolving towards the delivery of radiation with higher DRs to improve oncological treatment. Knowledge of biological effects depending on the radiation patterns used is aimed at expanding therapeutic options in radiotherapy, at the same time contributing to the reduction of radiation-related complications. The results achieved in this work are in line with recent research on the dose-rate effect.

It can be concluded that this experiment can have practical significance. In future practice, it may be possible to shorten the exposure time, increasing patient's comfort during radiotherapeutic treatment. Further research in this direction may contribute to the improvement of radiotherapy, because each subtle change in radiation therapy is very significant for the patients and their further life comfort.

Conflict of interest

No conflict of interest

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