Cellular response to proton radiation

Author: Anna Michaelidesová

Abstract

The thesis examines selected aspects of the biological effectiveness of clinical proton beams. The effectiveness of protons was studied using cell cultures and several biological endpoints, such as clonogenic cell survival, apoptosis induction, micronuclei formation, and formation of double strand breaks. Next to the estimation of the relative biological effectiveness along a spread-out Bragg peak in single and fractionated regimes, the bystander effect and the comparison of passive and active irradiation modes are given.

Introduction

Clinical advantages of a proton beam were first suggested by Wilson in 1946 in his paper about the radiological use of high energy protons [1]. Since the implementation of protons for cancer treatment in 1954 [2], an increased interest in proton therapy has been observed worldwide. The achievement of an optimal Relative Biological effectiveness (RBE) for proton therapy is a very discussed topic and uncertainties in RBE have been considered as a potential cause of radionecrosis in proton therapy patients [3]. The RBE of a studied radiation is the ratio of the absorbed dose of a reference radiation to the absorbed dose of the studied radiation that induce the same level of biological effect.

The aim of the present study was to investigate several aspects of the biological effectiveness of clinical proton beams. Firstly, a comparison between a clinical and a non-clinical proton facility is given. For the needs of the thesis, the Bystander effect was also investigated to find out if the cell medium has to be changed or not after the irradiations. Then, the RBE in four different positions of a Spread-Out Bragg Peak (SOBP) was estimated for several fractionation schemes. Next to this, in the last years, there is an increased interest in using active modes of proton therapy, due to the possibility of more conformal dose distributions in patients. Another advantage of active modes is the much lower secondary-induced radiation (mostly neutrons) from the components of the technological constructions or patient specific devices (collimators, compensators). The issue of the comparison of passive and active proton beams is due to this increased interest in active beams very actual and was also investigated.

In-vitro study at two proton facilities

Different proton facilities can generate similar proton beams; thus their parameters or biological effects do not have to be exactly the same. The aim of this part of the thesis was to estimate RBE values from three biological endpoints (cell survival, percentage of binuclear cells containing micronuclei (MN), and MN frequencies) in comparison to ^{60}Co γ-rays, and to discuss their applicability for the rest of the thesis. Two types of proton beam were used for the execution of the proton irradiations, thus a comparison between proton facilities was also possible.

The cells samples consisted of Normal human neonatal dermal fibroblasts (NHNDF) cell monolayers in T25 tissue flasks. The proton irradiations were held at the U120-M facility at the Center of Accelerators and Nuclear Analytical Methods situated in the Nuclear Physics Institute of the CAS in Rez, Czech Republic (CANAM infrastructure, project No. LM2011019) and at the Proton Therapy Center Czech in Prague (PTC), Czech Republic. The third and last part of samples was irradiated by a ^{60}Co γ-rays source at the Authorized Metrology Center of the Nuclear Physics

Institute of the CAS, in Prague, Czech Republic. This set of samples was used for comparison with protons.

From this part of thesis, a conclusion that RBE from various biological endpoints is an inappropriate method for evaluating the differences of two types of radiation. The author thinks that the best way to compare two radiation types is the cell survival and the other assays can be used for the estimation of additional parameters leading to knowledge about the complexity of the cell damage. Comparing the data from the two proton facilities, no statistically significant differences between them were found.

The bystander effect

The bystander effect is a term used for the situation when non-irradiated cells in the proximity of irradiated cells act as irradiated (cell death, mutation, chromosomal aberrations, long-term genomic instability) [4], [5]. In this part of thesis the author with her colleague Petra Sykorova used the medium transfer method [6] to observe the bystander effect in cells irradiated by γ–rays and proton radiation. The main goal was to find, if there are differences between the percentage of bystandered cells between the two types of radiation.

In the case of this study, using a fibroblast cell line an effect of medium transfer from irradiated cells was recognized. There was not observed any significant dose-depended effect in the bystandered cells, nor any significant difference in the percentage of bystandered cells between γ-rays and protons.

Relative biological effectiveness in several positions of a SOBP

The International Commission on Radiation Units and Measurements (ICRU) has recommended the use of a generic RBE value equal to 1.1 in the whole range of proton therapy [7] and most of the proton therapy centers around the world have adopted this value.

This recommended value is based on experimental studies held in-vitro and in-vivo in the early days of proton therapy using passive scattering modes. From the in-vitro studies, mostly performed on Chinese Hamster cell lines placed in the middle of SOBP, a mean RBE of 1.22 ± 0.02. The RBE from in-vivo experiments (mid-SOBP) had a mean of 1.10 ± 0.01 [8].

Recent studies show that the RBE is not constant, and it varies depending on a wide range of parameters, such as the initial beam energy, dose per fraction, position in the SOBP, cell line or tissue, and the studied biological endpoint [9].

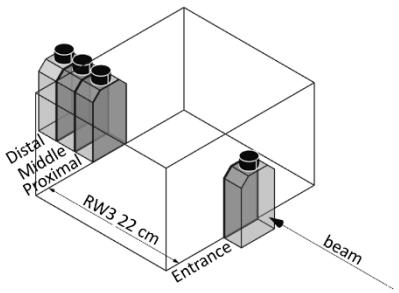


Figure 1: Irradiation setup

In the present study Normal human neonatal dermal fibroblasts (NHNDF) and Normal human skin fibroblasts (AG01522) were used as cell monolayers in T25 tissue flasks for proton irradiations. The irradiation setup is shown in figure 1. One sample was at the entrance of the beam and three in the homogeneous region of the SOBP in proximal, middle, and distal position. The irradiation plans were prepared using the treatment planning system XIO by ELEKTA at the Proton Therapy Center Czech, where the irradiations took place by several doses.

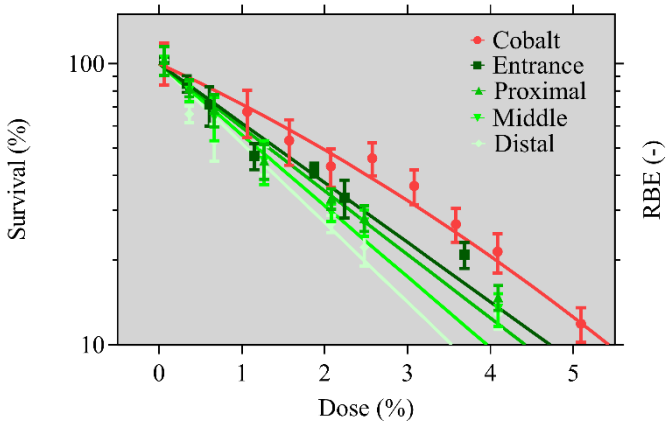


Figure 2: Cell survival (points) for 60-Cobalt and protons in different positions along SOBP together with curves resulting from the LQ model (lines).

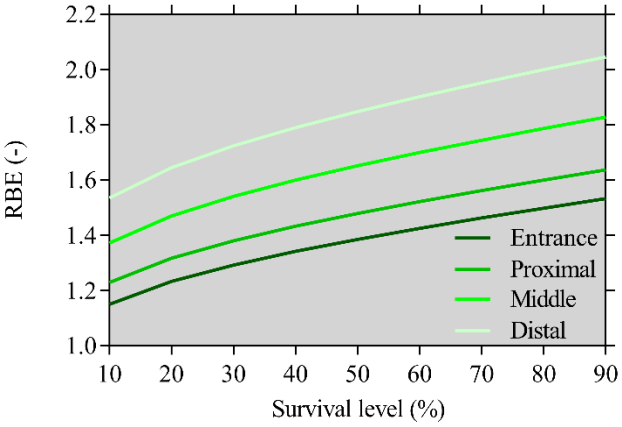


Figure 3: RBE values at different survival levels for the four positions in the SOBP.

The various positions were created using RW3 plastic plates, which are almost water equivalent. After the irradiation, cells were reseeded for cell survival assay and analyzed using the Linear-Quadratic model (LQ model). In the case of the single fraction irradiations using the NHNDF cells were also reseeded for the micronuclei assay.

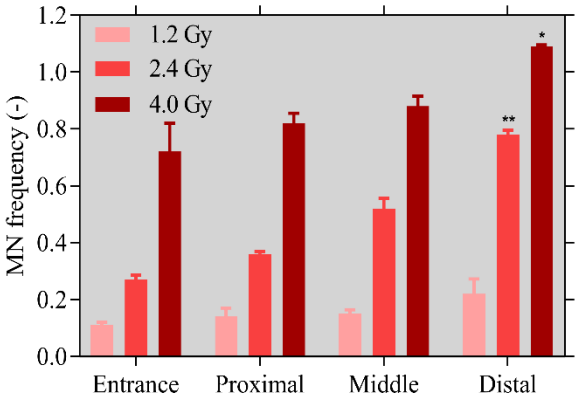
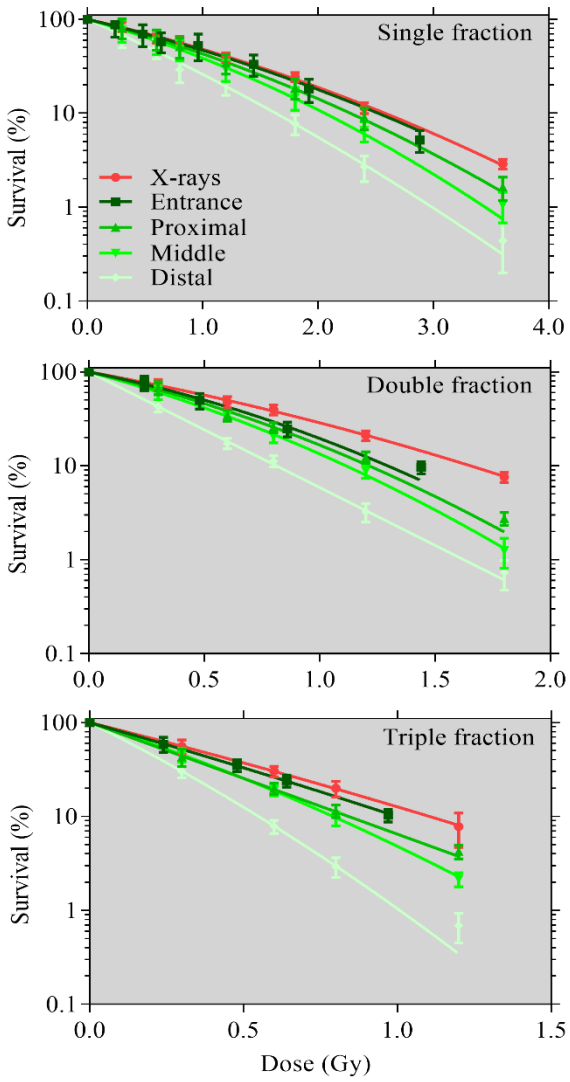


Figure 4: MN frequencies for three different doses in the four irradiation positions in the SOBP. The asterisks correspond to the two tailed Student's T-test (* P <0.05; ** P < 0.01) between proximal and middle or distal position.

In figure 2, the cell survival data for 60-Cobalt, as the reference radiation for the RBE calculations, and for the proton irradiations at the four irradiation positions are presented. The data were fitted using the LQ model. Using these fits, the RBE values in several cell survival levels were calculated and are shown in figure 3.

The estimation of the percentage of cells that have been seriously injured by proton radiation was held using the micronuclei assay. The micronuclei (MN) frequencies for three different irradiation doses at the four irradiation positions are shown in figure 4. By the MN frequency is meant the total number of micronuclei divided by the number of scored binuclear cells. An increasing complexity of chromosomal DNA damage is observed towards the distal parts of the SOBP, where the LET values were found to be higher using the Monte Carlo simulations.



Single, double, and triple fractionation schemes were studied using AG01522 cells to see effects of the fractionation to RBE in comparison to X-rays. The cell survival data fitted by the LQ model are shown in figure 5. The estimated RBE values from these survival data were ranging from 1.02 ± 0.15 for the single fraction irradiation scheme at the entrance position to 2.05 ± 0.08 for the triple fractional irradiation scheme at the distal position.

In most of the studied cases (fractionation scheme, survival level), the RBE values were found to be higher than the recommended by ICRU value equal to 1.1. The most significant difference is at the distal position of the SOBP. The obtained RBE values are decreasing with lower survival level (increasing dose) as expected. It seems that fractionation leads to even higher RBE values due to the recovery, redistribution and repopulation of the cells.

Figure 5: Cell survival for different fractionation schemes presented as points and their fit by the LQ model represented as solid lines. Dose presented in the graphs is the dose per fraction.

Comparison of active and passive modes

The issue of the comparison of passive and active proton beams is due to the increased interest in active beams very actual. Monte Carlo simulations on patients showed increased LET values at the distal fall-off of the proton beams for active beams compared to passive beams, which affects the biological response of tissues situated during proton therapy in this position [10].

Next to the reported higher LET values at the distal fall-off, the dose rates using active proton beams are much higher than in case of passive scattering. In the case of passive modes, the dose rate is a few Gy/min and in the case of active beams the dose rate inside a spot is around kGy/s (information provided by the technical staff of IBA at the PTC).

In the present study, medulloblastoma cell line DAOY was irradiated at two positions (proximal-1 and peak-2) by pencil beam scanning mode (PBS) as an active mode and double scattering (DS) as a passive mode. After the irradiations several biological endpoints were

followed (cell survival, apoptosis induction, DNA double strand breaks induction and the micronuclei assay). In figure 6, the cell survival is shown.

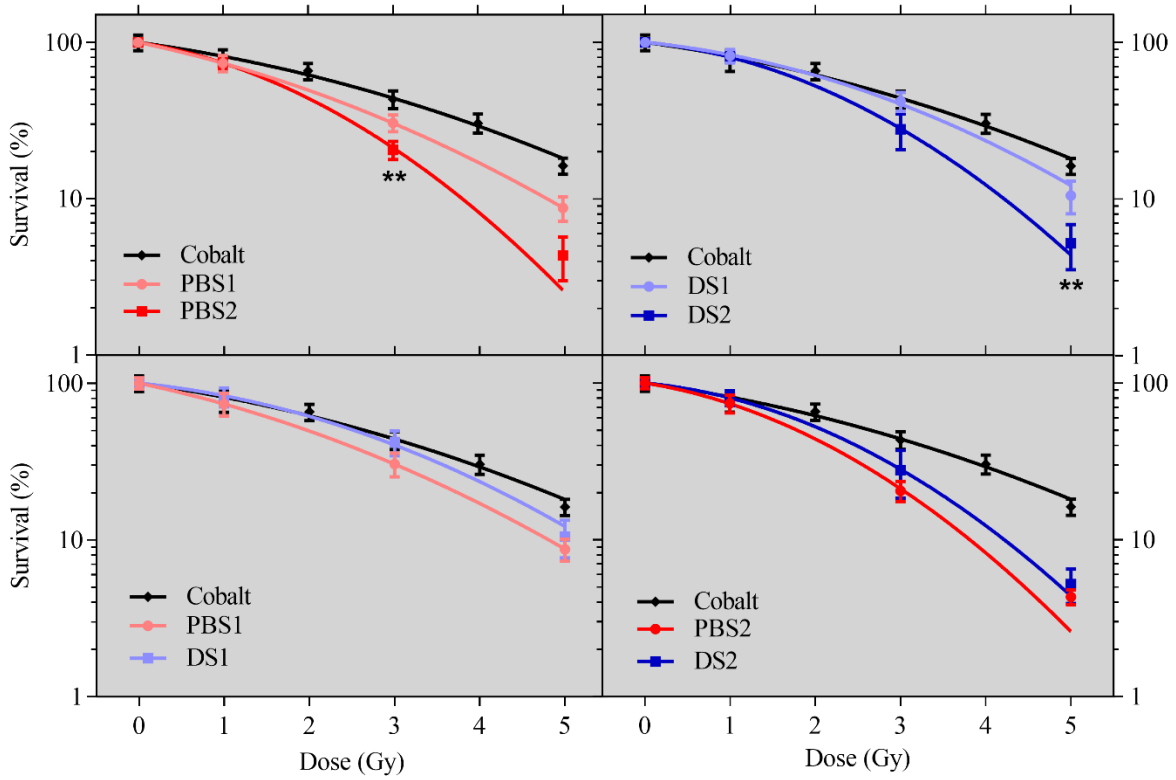


Figure 6: Cell survival of cells irradiated by PBS and DS modes in the two irradiation positions. On the upper side of the figure the comparison of the two positions for each of the modes is shown and on the lower side the comparison of the two modes for each of the positions.

As is shown in figure 6, the cell survival is for both positions lower for DS in comparison to PBS. Slightly more effective was PBS found also in the case of the other studied endpoints, but this slight difference was not found to be statistically significant, which in agreement with the study of Iwata [11].

Conclusions

In general, the RBE values found in the presented studies are in agreement with other published data and they differ from the recommended generic RBE value equal to 1.1 by the ICRU. Combination of the presented results and other published RBE values led to changes in the principles of the treatment planning of patients at the Proton Therapy Center Czech.

Based on the observations of higher RBE values at the distal parts of the SOBP, the number of single field irradiation plans was decreased, and in cases that the single field method is beneficial, due to the tumor's position, a reduction of the delivered dose is made at the few last millimeters of the treatment volume.

Another result of the RBE observations was that in case of two opposing treatment fields the depth-dose profiles are forced to be sloped with a decreasing physical dose along the SOBP, which leads to the compensation of the higher RBE values at the distal parts of the SOBP. Important is also to have in mind that it is better not to direct the beam to the critical organs, when it is possible, to minimize the probability of their damage.

References

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