Influence of 6 MV and 20 MV X- radiation dose rate on in vitro survive of the K-562 cell line

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ABSTRACT

BACKGROUND: Analysis of the survival rate of cells after irradiation with a specified dose of X-radiation might be one of the basic foundations for assessment of biological implications of ionizing radiation. Investigation of the influence of X-radiation dose rate on cells was carried out in vitro using the SF2 test.

AIM: The aim of this study was to investigate the influence of X-radiation dose rate on the surviving fraction of the K-562 cell line for two photon energies of 6 MV and 20 MV.

MATERIALS/METHODS: To measure the cells' reaction to X-radiation of variable dose rate human leukaemic K-562 cells were used. In order to fulfil the main aim of the study, the cell line was subjected to irradiation at two different dose rates. Total dose applied at once was 2 Gy. A quantitative evaluation of cell survival rate was carried out at every step of the experiment using a clonogenic assay.

RESULTS: High dose rate at the energy of 6 MV decreased the percentage of surviving cells to 23%, while lower dose rate decreased it only to 36%. A similar effect is observed at the energy of 20MV -namely at the higher dose rate the percentage of surviving cells is 18%, whereas at the lower one it is only 34%.

CONCLUSIONS: The experiment has shown that when using a lower dose rate, the biological effect of ionizing radiation is less pronounced. However, at a higher dose rate higher radiosensitivity of cells is observed.

KEY WORDS: K-562 cells; SF2 test; radiosensitivity of cells; clonogenic assay

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BACKGROUND

The research on the influence of radiation on cells might be carried out in vitro, when using SF2 test. This test consists in quantitative analysis of cells survival rate after irradiation with a test dose of 2 Gy [1, 2]. The term "cell survival" means maintenance of the capacity for the production of unlimited number of progeny cells through proliferation. Thus SF2 test practically means calculating the proportion of population which retains the ability of reproduction after irradiation, namely the ability to form colonies comprising at least 50 cells during the period of time corresponding to duration of 5–6 cell divisions. Additionally it is assumed that every colony evolves from a single cell, therefore number of colonies is equal to the number of cells surviving given radiation dose. The number of colonies to the number of cells present in the population before irradiation ratio determines the surviving fraction (SF) [3, 4, 5].

AIM

The aim of this study was to investigate the influence of X-radiation dose rate on the surviving fraction of K-562 cell line for two photon energy of 6 MV and 20 MV. Detailed tasks encompassed elaboration of a method for cells

irradiation and elaboration a method for determination of the surviving fraction after applied dose of radiation.

MATERIALS AND METHODS

To measure the cells reaction to X-radiation at variable dose rate K-562 cancer cell line was used. K-562 is an erythroleukemic cell line causing acute myelogenous leukemia. K-562 cells are grown in suspension cultures in laboratories. These cells are non-adherent, which means that they do not stick to the inner surface of the plate, but they grow as a cell suspension in the culture medium. They constitute a relatively homogenous population (Figure 1) [6, 7, 8].

K-562 cell line used in the experiment was received from the Department of Diagnostic and Cancer Immunology in Great Poland Cancer Center in Poznan. Originally K-562 cells were isolated from a 53-year-old patient with chronic myelogenous leukemia in terminal blast crises [8].

In the initial phase of the experiment, in order to create a survival curve for K-562 cells and evaluate their radiosensitivity, the cells were irradiated with radiation dose between 1 and 8 Gy (multiples of 1 Gy) at 3 Gy/min dose rate and of 6 MV energy. The relationship between the absorbed radiation dose and surviving fraction was established with the use of semi-logarithmic coordinate system, which provided the survival curve. Relying upon it the extrapolation number – n and the mean value of lethal dose – D_0, which decreases the percentage of surviving cells to 37%, were determined [9].

Subsequently, in order to fulfill the main aim of the study, the cell line was subjected to irradiation at two different dose rates (0,6 and 0,3 Gy/min). The energy of photon radiation used was 6 MV and 20 MV. The total dose applied at once was 2 Gy. The duration of irradiation was calculated with reference to Varian Eclipse treatment planning system. Test-tubes with the cells placed on the stand were exposed to radiation in the PTW Freiburg water phantom at the depth of 5 cm for 6 MV energy and 10 cm for 20 MV energy under the following reference conditions: area – 10 x 10 cm^2, gantry, couch and collimator angles – 0°, source to skin distance (SSD) – 100 cm. The measurements were done with the use of Varian Clinac 2300 linear accelerator. In order to evaluate the reproducibility of results and subsequently calculate the mean values, the cells were irradiated in three seperate test-tubes during every single measurement. In addition three so called control test-tubes were not exposed to radiation, but in any other aspect they were treated in the same manner as the irradiated test-tubes [10].

A quantitative evaluation of cells survival was carried out at every step of the experiment with the use of clonogenic assay. Results obtained from every single measurement were compared to results gathered for the control samples (non-irradiated cells were stored in the same conditions as irradiated cells), and surviving fraction SF was calculated according to the formula:

$$SF = \frac{\text{Colonies counted}}{\text{Cells seeded} * \text{PE}/100}$$

PE – the plating efficiency for the nonirradiated cells was calculated by the following formula:

PE = (colonies counted/cells seeded) x100% [11].

RESULTS

In the initial phase of experiment the cells were irradiated with the dose range between 1–8 Gy (multiples of 1 Gy). Determined mean values of surviving fraction were compared to absorbed radiation dose in a semi-logarithmic coordinate system, which in consequence led to obtaining the survival curve. Extrapolation of the exponential part of the curve to the crossing point at axis of ordinates provided an extrapolation number n = 1,13, which is the

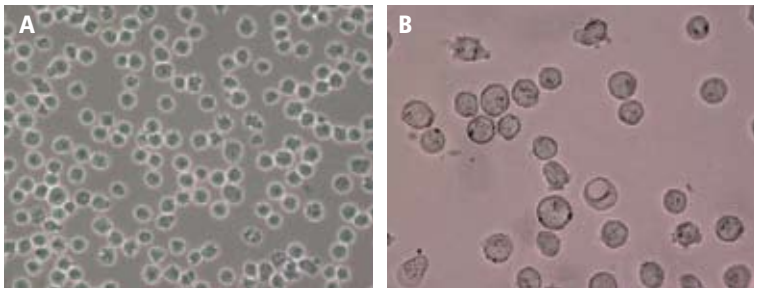

Fig. 1. K-562 cells. a) 10x /0,25 magnification; b) 20x /0,40 magnification. (The photograph was taken with Nikon Eclipse TS100 microscope.)

measure of targets number mean value in the analyzed population. The mean value of lethal dose (D_0), SF = – namely a dose which decreases the proportion of surviving cells to 37%, measured within rectilinear part of the curve was D_0 = 1,22 Gy. D_0 = 1,22 Gy and n = 1,13 values (at 3 Gy/min and of 6 MV energy) are similar to the survival values of acute lymphocytic leukemia cells which ranks to high radiosensitivity cells [9].

The reaction of K-562 cells in response to radiation of variable dose rate is depicted in figure 2 (for 6 MV energy) and figure 3 (for 20 MV energy). The results are shown in the bar charts. The length of every single bar is proportional to the mean value of surviving fraction for 2 Gy dose at a specified dose rate. Values of errors (measurement uncertainties) are shown as vertical lines.

High dose rate (3 Gy/min) at 6 MV energy decreased the percentage of surviving cells to 23%, whilst lower dose rate (0,6 Gy/min) only to 36%. A similar effect occurs for 20 MV energy, namely at the higher dose rate the percentage of surviving cells amounts to 18%, whereas at the lower dose rate it is only 34% (Figure 3).

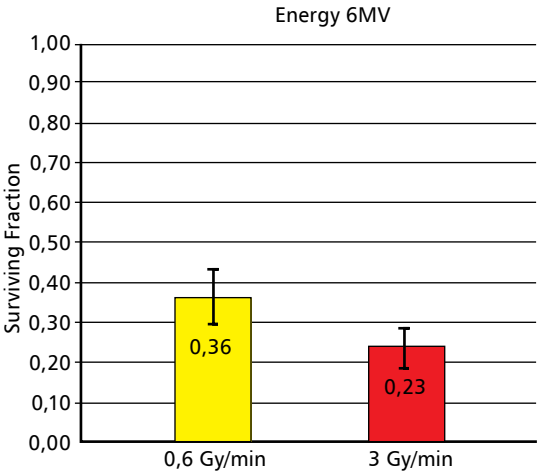

Fig. 2. Influence of dose rate on survival rate of K-562 cells for X electromagnetic radiation of 6 MV photon energy.

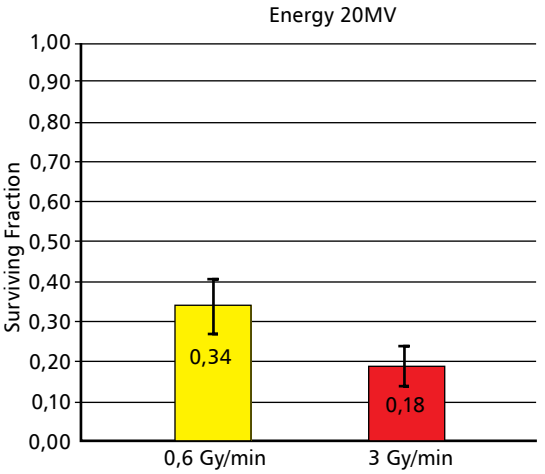

Fig. 3. Influence of dose rate on survival rate of K-562 cells for X electromagnetic radiation of 20 MV photon energy.

DISCUSSION

K-562 cell line was subjected to irradiation of two different dose rates (0,6 and 0,3 Gy/min), for which X-radiation of 6 MV and 20 MV photon energy was used. The total dose applied at once was 2 Gy. The experiment has shown that in the case of using of lower dose- rate radiation (0,6 Gy/min) and applying it during longer periods of time, the biological effect of ionizing radiation is less pronounced. However at the higher dose rate (3 Gy/min) higher radiosensitivity of cells is observed. High dose rate (3 Gy/min) at the energy of 6 MV decreased the percentage of surviving cells to 23%, while lower dose rate (0,6 Gy/min) decreased it only to 36%. A similar effect is observed at the energy of 20 MV – namely at the higher dose rate the percentage of surviving cells amounts to 18%, whereas at the lower one it is only 34%. The observed difference might be due to postirradiation damage repair processes occurring in the cells. At higher dose rates the rate of damage emergence is proportionally high, so the capacity for effective damage repair decreases and in consequence less cells survive.

The above-mentioned assertions are a result of experiments carried out with cells cultured *in vitro*. *In vitro* culture enables to observe what happens to separate single cells, which is not possible *in vivo*. Therefore it is interesting whether K-562 cells react to the radiation in the same manner *in vivo*, as they do in the culture or not. Addressing this question is problematic because of numerous doubts. *In vitro* system is too simplified compared to complexity of an organism. Cells in an organ-

ism do not exist in isolation from each other, therefore they are not able to maintain self-efficiency in the culture and that is why they are supplied with an environment, which to some extent substitutes plasma or extracellular matrix. Moreover cells cultured in vitro are isolated from other types of cells with which they naturally interact within a given system. Therefore there is an undoubted need for research, including clinical trials, which could address so many questions related to radiosensitivity of cells. Obtained results cannot be correlated with clinical effects of irradiation, however they might set a solid foundation for further experiments.

CONCLUSIONS

1. A quantitative cell survival analysis of K-562 cell line exposed to radiation might be carried out *in vitro* with the use of clonogenic assay.

2. The mean value of lethal dose D_0=1,22 Gy and extrapolation number n=1,13 suggest a high radiosensitivity of K-562 cells.

3. The X-radiation dose rate has a significant influence on survival rate of K-562 cells cultured in vitro, at the higher dose rate less cells survive.

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