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

Mariusz GRUDA, Grażyna KOSICKA, Ewelina KONSTANTY, Dariusz KOWALCZYK

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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Greater Poland Cancer Centre

Address for correspondence:
Mariusz Gruda
Greater Poland Cancer Centre
Garbary 15
61-866 Poznań
Poland
e-mail: grudamr@gmail

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

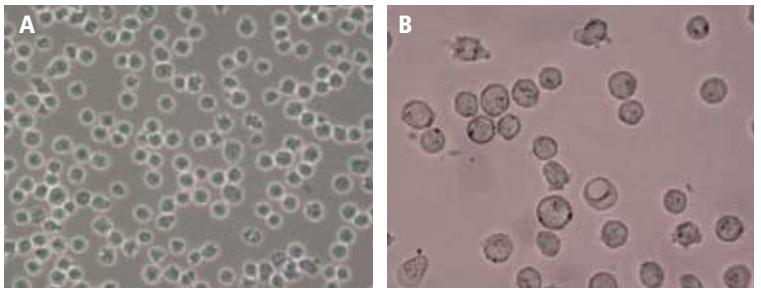

Fig. 1. K-562 cells. a) 10x /0,25 magnification; b) 20x /0,40 magnification. (The photograph was taken with Nikon Eclipse TS100 microscope.)

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