INTRODUCTION

Radiotherapy is a conventional localized cancer treatment method using ionizing radiation. Interaction of high energy photons with biological tissue results in production of the reactive oxygen species (ROS), that in turn may cause significant cellular damage. Depending on the X-ray energy delivered to the irradiated cells four scenarios are possible: 1) No significant changes in cell viability at low irradiation doses; 2) Significant damages in irradiated cells caused by radiation induced ROS that may lead to temporary mitotic arrest at the G1/S or G2/M stages of the cell cycle at high doses; 3) Initiation of the apoptotic cell death due to severe ROS damage; 4) Necrosis of cells because of colossal unreparable damage done by radiation generated ROS.

It is known that the application of 2 Gy per fraction for cancer treatment is sufficient enough for effective balancing between tumor destruction and possible low health tissue complications. Due to the fact that 2 Gy irradiation most likely is responsible for the apoptotic cancer cell death, significantly less inflammation and other systemic response is introduced, as compared to necrotic cell death. Also other cellular reactions to irradiation are possible, e.g. radiation induced continuous proliferation of tumor cells. Moreover, cells that survive significant DNA damage and proliferate can induce secondary, non-metastatic, tumor.

In the performed research radiation induced ROS generation, cellular DNA damage and exposed cell viability were investigated, and irradiation dose related complex cellular response was discussed. We also demonstrated cellular response to different irradiation doses in form of ROS production and mitotic arrest. Performed analysis of the results led to suggestion of an irradiation dose threshold between 2 Gy and 4 Gy, which differentiates between the stages of cell response to irradiation. Presence of a threshold was approved by the results of DNA damage evaluation, assessment of post irradiative ROS production, relative mitotic arrest in cells and cell viability investigations.

MATERIALS AND METHODS

In order to perform complex numerical evaluation of radiation impact on the processes that contribute to the damage of DNA, cells were irradiated by 6 MeV X-ray photons to different doses (2-10 Gy). The increase of ROS generated inside the cell and in the medium, cell DNA damage, cell viability and relative mitotic arrest was evaluated after irradiation of cells.

Chinese hamster ovary cells (CHO-K1) were cultured at 37°C in water-saturated air containing 5% CO2. The cells were routinely passaged in growth medium supplemented with 10% of fetal calf serum. The cell doubling time was approximately 12 h.

Irradiation of cells was performed in linear accelerator Varian Clinac DMX using 6 MeV X-ray photons. The treatment dose delivery rate to the target of 3 Gy/min was applied. The irradiation dose varied between 0.5 and 10.0 Gy. Irradiation was performed in broad beam geometry using 10x10 cm² irradiation field. Petri dish (Ø25 x 10 mm) with cells was placed in a special cavity located at 6 cm depth of a (30 x 30 x 11) cm³ PMMA phantom. Dose simulation was performed using Varian Aria AAA algorithm keeping 100% isodose at the depth of 6 cm.

Clonogenic cell assay was used to assess radiation induced effects in irradiated cells. Cell colonies were manually counted using ImageJ software plug-in Colony Counter. Colony residence area was assessed using ImageJ software plug-in Analyze Particles, which enabled analysis of the scanned image of Petri dish with cells detecting separate cell colonies and calculating the average area of colonies within the dish.

RESULTS AND DISCUSSION

Generation of ROS in CHO cell cultures (10⁶ cells/ml with added 50 µM/ml DCFH-DA dye for the evaluation) irradiated to the doses from the interval of 1 Gy to 8 Gy were investigated 1 hour after irradiation. The results of dose dependent DCF fluorescence intensity changes in irradiated cells are provided in Fig.1.

CONCLUSIONS

Based on performed analysis time dependent dose threshold of 2-4 Gy for maximal delayed mitosis effect in irradiated cells has been set. Presence of such a threshold was approved by the results on DNA damage evaluation, post irradiative ROS production, cell viability and partly by data on relative mitotic arrest in irradiated cells indicating that application of dose threshold based irradiation model can contribute to the improvement of fractionated radiotherapy treatment efficiency.

REFERENCES