THE EVALUATION OF RELATIVE ROS GENERATION IN MEDIA AND CELLS TRIGGERED BY SCATTERED DOSE AFTER X-RAY IRRADIATION

Tadas Didvalis[1,2], Paulius Ruzgys[1], Saulius Šatkauskas[1], Diana Adlienė[3], Saulius Mickevičius[1]

[1]Vytautas Magnus University, K. Donelaičio 58, LT-44248, Kaunas, Lithuania
[2]Hospital of Lithuanian University of Health Sciences Kauno klinikos,Eivenių 2, LT-50161, Kaunas, Lithuania
[3]Kaunas University of Technology, K. Donelaičio 73, LT-44249, Kaunas, Lithuania
tadas.didvalis@fc.vdu.lt

Radiotherapy is conventional type of cancer treatment. However, a fundamental research on ionising radiation application to cells and tissues is still in need.. The rise of complex and multi-field radiotherapy techniques, such as IMRT, VMAT and SRS usage in patient treatment, suggests that the evaluation of low doses impact for healthy and malignant tissue is in need[1]. In this study we present a simulation of scattered radiation doses, and experimentally obtained relative ROS generation with and without side scattering effect in affected media and in cells.

Materials and methods. We compare obtained modeling results with ionizing radiation induced ROS generation by irradiating 96 well plate with and without water between plate wells. Water works as scattering material between individual wells and, in addition to the primary photons from the radiation source, increase the number of scattered and secondary photons in a medium. For this study black flat bottom 96 well plate (Thermo Fisher) was placed in a laboratory made PMMA phantom with 4 cm of build-up plastic below and above plate. We applied ionizing radiation dose of 8 Gy with a linear accelerator Varian Clinac DMX with 6 MeV energy X-ray photons and 4x4 cm^2 irradiation field. This field size allows to fully covers 36 wells. Linear accelerator gantry was positioned from the bottom of the phantom to reduce dose distribution distortions induced by air gaps above medium. Monitor unit calculation and dose distribution simulation were performed using AAA algorithm on Varian Eclipse treatment planning system on computed tomography scans of phantom withplate with scatter material and without scatter material. Obtained values for in-field and out-of-field doses were used for comparison with experimental results of this study. Chinese hamster ovary cells (CHO-K1) were used for this study. Our recent study [2] showed that one of the parameters that can be monitored and correlates to cell DNA damage and cell death is ROS generation during and after irradiation, therefore we used DCFDA dye method for ROS evaluation. Cells were incubated with 50 µM/ml of 2',7'-dichlorodihydrofluorescein diacetate (H_2DCF-DA) molecules for 60 min prior to the irradiation and then distributed in each well. 50 µl per well). After irradiation the amount of 50 µl of 96 % ethanol was put on cell suspension in order to equally disperse DCF dye. After 30 min incubation the DCF induced fluorescence was measured by using spectrophotometer (TECAN Genios Pro 96/384). For the experiments with additional scattering material 100 µl of water were added between each well.

Results: We found a significant ($p < 0.001$) increase of generated ROS in wells with scatter material in both in-field and out-of-field wells by 28.18 % for in-field and by 45.07 % for out-of-field wells. It is notable that scatter material increases relative in-field and out-of-field ROS concentration by 4.6 %, from 34.95 % to 39.56 %. The experimental results go with an agreement with dose distribution simulation.

Conclusions: Side scattering of an applied x-ray energy is significantly changing applied energy to affected cells in the in-field and out-of-field cells, thus in turn ROS generation is altered. It is known that cell death after X-Ray irradiation is a result of DNA damage generated by induction of ROS. Therefore, a ROS generation evaluation in out of irradiation field is crucial for out-of -field cell viability change estimations. Here we show a significant ROS generation in out-of -field for the viability change of the affected cells.

[1] G. Lazzari, A. Terlizzi, MG Leo, G. Silvano, VMAT radiation-induced nausea and vomiting in adjuvant breast cancer radiotherapy : The incidental effect of low-dose bath exposure, Clin Transl Radiat Oncol, 43–8 (2017).
[2] T. Didvalis, P. Ruzgys, S. Šatkauskas et al., Assessment of ROS production using DCFH-DA dye in CHO cells after application of ionizing radiation, 2nd International Conference SmartBio 3th – 5th May 2018, 34 (2018).