

The Usefulness of Premature Chromosome Condensation Scoring Module in Cell Response to Ionizing Radiation

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Abstract : Due to the mitotic delay, poor mitotic index and disappearance of lymphocytes from peripheral blood circulation, assessing the DNA damage after high dose exposure is less effective. Conventional chromosome aberration analysis or cytokinesis-blocked micronucleus assay do not provide an accurate dose estimation or radiosensitivity prediction in doses higher than 6.0 Gy. For this reason, there is a need to establish reliable methods allowing analysis of biological effects after exposure in high dose range i.e., during particle radiotherapy. Lately, Premature Chromosome Condensation (PCC) has become an important method in high dose biodosimetry and a promising treatment modality to cancer patients. The aim of the study was to evaluate the usefulness of drug-induced PCC scoring procedure in an experimental mode, where 100 G2/M cells were analyzed in different dose ranges. To test the consistency of obtained results, scoring was performed by 3 independent persons in the same mode and following identical scoring criteria. Whole-body exposure was simulated in an in vitro experiment by irradiating whole blood collected from healthy donors with 60 MeV protons and 250 keV X-rays, in the range of 4.0 – 20.0 Gy. Drug-induced PCC assay was performed on human peripheral blood lymphocytes (HPBL) isolated after in vitro exposure. Cells were cultured for 48 hours with PHA. Then to achieve premature condensation, calyculin A was added. After Giemsa staining, chromosome spreads were photographed and manually analyzed by scorers. The dose-effect curves were derived by counting the excess chromosome fragments. The results indicated adequate dose estimates for the whole-body exposure scenario in the high dose range for both studied types of radiation. Moreover, compared results revealed no significant differences between scores, which has an important meaning in reducing the analysis time. These investigations were conducted as a part of an extended examination of 60 MeV protons from AIC-144 isochronous cyclotron, at the Institute of Nuclear Physics in Kraków, Poland (IFJ PAN) by cytogenetic and molecular methods and were partially supported by grant DEC-2013/09/D/NZ7/00324 from the National Science Centre, Poland.

Keywords : cell response to radiation exposure, drug induced premature chromosome condensation, premature chromosome condensation procedure, proton therapy

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