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The study of DNA damage in lymphocytes and granulocytes after whole body irradiation in mice

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Whole body ionizing gamma irradiation represents genotoxic stress, which has a decisive influence on all blood cell lines. Lymphocytes belong to the most radiosensitive cell lineages. After exposure of the organism to ionizing irradiation they die by apoptosis, the extent of which depends on the reeived dose. Bone marrow contains a reserve pool of functionally mature granulocytes, which might be readily mobilized into peripheral blood. While peripheral blood lymphocytes represent one of the most sensitive cellular tools for biodosimetric studies, granulocytes have not been in the focus of biodosimetric studies so far. We have compared a biodosimetric potential of peripheral blood and bone marrow lymphocytes and granulocytes by quantifying γ-H2AX expression.

Material and Methods: Cells were isolated from the heparinized peripheral blood and bone marrow from sham-treated control and irradiated female mice (doses of 1-3-5-7-9 Gy) one hour after treatment. The level of histon H2AX phosporylation was detected by FITC-conjugated anti-phospho histone H2AX (Ser139) monoclonal antibody (Millipore, USA) in lymphocytes (lymphogate in the FSC/SSC dotplot and CD45+Gr1- surface phenotype) and granulocytes (CD45+GR1+ cells with higher SSC parameter) by surface immunophenotyping followed by intranuclear staining and flow cytometry. CyAn flow cytometric analyzer (Beckman Coulter) and Summit 3.4 software were used for data acquisition and analysis.

Results: At lower doses (1-7 Gy), the intensity of H2AX phosphorylation appears to be higher in the granulocyte population than in lymphocytes both in peripheral blood and bone marrow. At higher does (9 Gy), H2AX is phosporylated to the same degree in both of major leukocyte populations studied.

Conclusions: Fast biomarkers associated with DNA damage like γH2AX offer an opportunity to evaluate the received dose immediately after irradiation. Blood lymphocytes have commonly been used for visualization of double strand breaks (DBS) early after radiation exposure. We have shown that granulocytes are as good biomarker as their lymphoid counterparts, the granulocyte population appears to be even more sensitive at lower doses. In addition, the use of granulocytes for the mean of cellular fluorescence of γ-H2AX could be profitable at higher doses of irradiation, when the number of lymphocytes is severely reduced.

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