

SUMMARY

We proved that all studied cells of blood system die after irradiation by apoptosis. We used immortalized tumor cell lines HL-60 and MOLT-4, hematopoietic stem cells and peripheral blood lymphocytes.

Apoptosis was detected by DNA content analysis (subG₁ peak), evaluation of mitochondrial membrane antigen APO2.7, and detection of phosphatidylserine presence in outer cell membrane by Annexin V.

HL-60 cells (human promyelocytic leukemia) are relatively dedifferentiated without functional protein p53. After irradiation by the doses up to 10 Gy apoptosis is induced in these cells after cell cycle arrest in G₂ phase (delayed apoptosis). Irradiation by higher doses induces fast apoptosis from the phase of the cycle in which the cells were irradiated.

The second model cell line was T lymphocytic leukemia MOLT-4. MOLT-4 cells express wild type of p53 and are very radiosensitive. We proved **dose-dependent increase in apoptosis induction 16 h after the irradiation by the doses 0.2-5 Gy by analysis of APO2.7 without permeabilization of the cells.**

In studies of apoptosis induction in human lymphocytes we used analysis of Annexin V and PI binding together with analysis of CD markers of particular lymphocyte subpopulations.

In the dose range 1-10 Gy we observed dose-dependent increase in A⁺ PBMC 16 h after in vitro gamma irradiation. We prove that relative abundance of A⁺/PI⁺ population does not change in time, these cells have short lifetime and apoptotic cells are accumulated in a late A⁺/PI⁺ phase. Amount of A⁺/PI⁺ cells increases in dose-dependant manners.

In subpopulation of live, A⁺/PI⁺ cells, we proved dose-dependent decrease in NK cells 16 h after the irradiation for dose range 1-10 Gy. For doses up to 3 Gy this dose-dependence is better 48 h after the irradiation.

NK cells can be classified into 2 subpopulations using density of CD56 marker - CD56^{high} and CD56^{low}. CD56^{high} subset is significantly more sensitive to ionizing radiation in comparison to CD56^{low} NK cells.

Studies of NK cells after in vivo irradiation of tumour patients is complicated, because apoptosis of NK cells is increased due to reaction to tumour cells.

In the group of patients treated for endometrial carcinoma by "box" irradiation (small volume in pelvis) the dose of 2 Gy induced only low decrease in NK cells. However, after whole-body irradiation (2 Gy) of patient treated for hematologic malignancy we proved a significant decrease of NK cells, mainly 48 h after the irradiation, which correlates with in vitro experiments.