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Comparison of Biological Effects of γ-Radiation of Low and Ultra-High Dose Rate on Lymphocytes and Cultured Human Malignant Lymphoma Cells

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We studied the effect of low and high-dose rate photon radiation on activation of cell death by apoptosis and necrosis in malignant cell lines of lymphocytic origin Raji and Jurkat (human B and T-cell lymphomas) and normal human lymphocytes from healthy volunteers. It was shown that photon radiation with ultra-high dose rate induced significantly higher levels of "early" apoptosis and lower levels of necrosis compared to γ-radiation with dose rate used for radiation therapy.

Key Words: *ultra-high dose rate γ-radiation; dose rate; apoptosis; lymphocytes; Raji and Jurkat cell culture*

The use of radiation therapy for the treatment of malignant tumors is based on the fundamental difference in radiosensitivity of normal and tumor cells/tissues, which leads to the so-called "therapeutic range" of dosages allowing destruction of the tumor tissue with minimum damage to healthy tissue. This difference is determined by molecular genetic characteristics of tumor cells including control of proliferation, apoptosis and, most important, DNA repair, and other physiological functions of cells [6,8].

Different characteristics of the used ionizing radiation can produce different effect on certain processes of cell damage and repair [1,4,7]. Thus, analysis of the effects of ionizing radiation with different physical characteristics on normal and tumor cells can reveal the most promising for medical practice regimens.

In this contest, the least studied is photon (γ) radiation with ultra-high dose rate; biological effects of this radiation attract increasing interest in recent years [5,9,10]. W have previously demonstrated that the effect of photon (γ) radiation, in particular, radiation with a dose rate of about 10^8 Gy/sec, has a number of specific biological effects in comparison with photon radiation with a dose rate of 0.01-0.1 Gy/sec [2,3]. In particular, it was shown that the levels of apoptosis and necrosis in peripheral blood lymphocytes isolated from healthy donors and in some cultured tumor cell lines differed considerably after irradiation with photon radiation with ultra-high dose rate in the range of therapeutic doses [2]. Therefore, we hypothesized that photon radiation can have different effects on normal and tumor cells.

Here we compared biological effects of photon radiation with ultra-high dose rate (Angara-5-1 unit) and low dose rate (Rokus-AM therapeutic unit) on lymphocytes and cultures of human malignant lymphoma cells Raji и Jurkat.

MATERIALS AND METHODS

We used suspensions of normal lymphocytes isolated from the blood of healthy donors. Mononuclear fraction was isolated as previously described [2]. Raji and Jurkat malignant lymphoma cells were cultured in RPMI-1640 (PanEco; No. C310) supplemented with 10% fetal calf serum (BioWest; No. S1800) by standard methods.

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The samples were irradiated using Angara-5-1 radiation unit and Rokus-AM therapeutic unit as described previously [2] in single doses of 1, 2, 3, and 4 Gy. The dose rate of radiation in Rokus-AM was about 1 Gy/min; the dose rate of ultra-high radiation at Angara-5-1 was 1-4×10^9 Gy/min.

After 24 h incubation, the proportion of apoptotic and necrotic cells in irradiated samples was determined by flow cytofluorometry. Two techniques were used: staining of native sample with Annexin V-FITC Kit (Beckman Coulter) containing annexin V and propidium iodide (PI) and PI staining of fixed sample. The level of early apoptosis was evaluated by the proportion of annexin V^+ cells and late apoptosis estimated by the proportion of the subdiploid peak in cell suspension stained with PI.

The analysis was performed on a Cytomics FC500 flow cytofluorometer (Beckman Coulter). The level of apoptosis was measured relative to the sample exposed to zero radiation dose (taken as 1).

The data were processed statistically using the parametric Student's *t* test (Statistica 13.0). The differences were significant at $p<0.05$.

RESULTS

The levels of apoptosis recorded as the value of the subdiploid peak in the cell suspension of normal and tumor lymphocytes stained with PI depending on the type and dose of photon radiation used (Fig. 1).

The level of apoptosis in all three types of analyzed cell suspensions increases with increasing the radiation dose. For Jurkat and Raji malignant lymphoma cells, the level of induced apoptosis was higher by 3 and 2 times, respectively. The level of apoptosis for tumor lines did not significantly depend on the type of irradiation.

Both types of photon radiation induced a higher level of apoptosis in lymphocytes of malignant tumors in comparison with normal donor lymphocytes

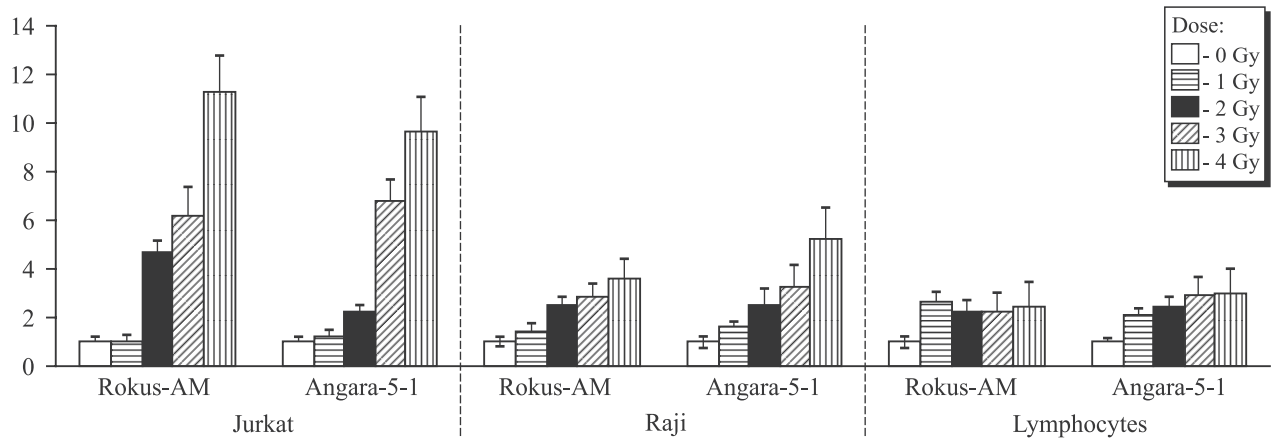

Fig. 1. Dose dependence of the intensity of late apoptosis induced by photon radiation of different dose rates in the suspension of lymphocytes from healthy donors and tumor lymphocytes of Raji and Jurkat cell lines. Ordinate: number of cells with hypodiploid DNA content (apoptotic bodies), %.

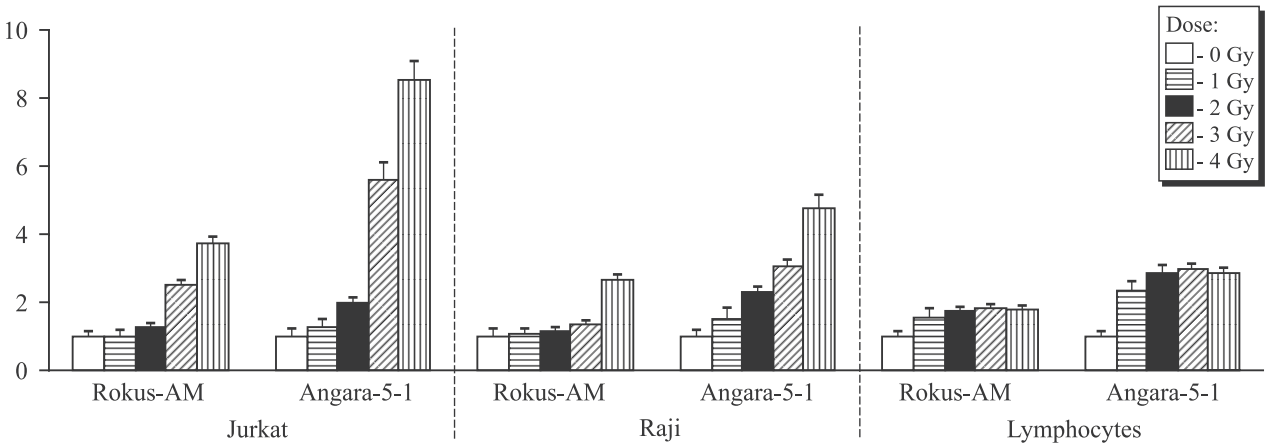

Fig. 2. Dose dependence of the intensity of early apoptosis induced by photon radiation of different dose rates in the suspension of lymphocytes from healthy donors and tumor lymphocytes of Raji and Jurkat cell lines. Ordinate: number of annexin$^+$ cells (apoptotic bodies), %.

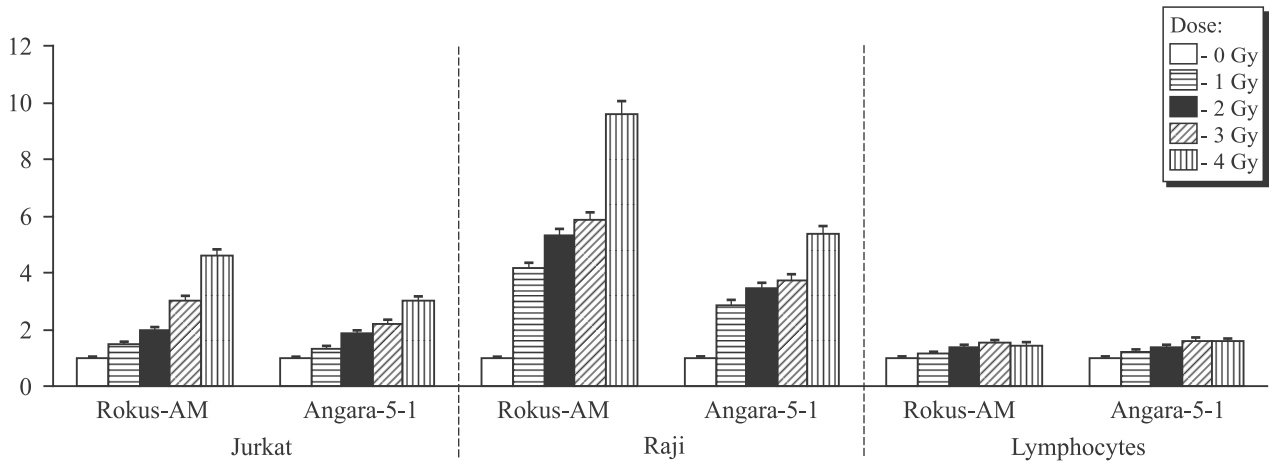

Fig. 3. Dose dependence of the intensity of necrosis induced by photon radiation of different dose rate depends on the dose in the suspension of lymphocytes from healthy donors and tumor lymphocytes of Raji and Jurkat cell lines. Ordinate: the number of cells stained with both annexin and PI (necrosis and late apoptosis), %.

(Fig. 2). The induction of early apoptosis in lymphocytes of malignant lymphomas was significantly higher when γ-radiation of ultra-high dose rate was used. It should be noted that the level of induced apoptosis was higher for Jurkat cells characterized by T-cell phenotype.

We also analyzed the level of activated necrosis estimated by the number of cells stained with both dyes (annexin and PI). The level of activated necrosis, similar to activated apoptosis, was significantly higher in tumor cells in comparison with normal lymphocytes. (Fig. 3). However, γ-radiation of ultra-high dose rate caused significantly lower levels of necrosis than radiation with low dose rate.

Thus, photon (γ) radiation induced death of normal lymphoid and tumor cells mainly via apoptosis, and the level of apoptosis induced in tumor cells was several times higher than in normal lymphocytes. The level of induced early apoptosis detected by annexin V binding was significantly higher when γ-radiation of ultra-high dose rate was used. Photon radiation of ultra-high dose rate causes significantly lower levels of necrosis compared to γ-radiation with dose rate used for radiation therapy.

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