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INDUCTION AND REPAIR OF DOUBLE-STRAND DNA BREAKS (DSBs) IN 5 CELL LINES AFTER SUBLETHAL DOSES OF X-RADIATION - USE OF PULSE-FIELD GEL ELECTROPHORESIS

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Among DNA lesions induced in cells by ionizing radiation, DSBs appear with the lowest frequency - approx. 20-30 DSBs per cell per Gy of X rays [1,2]. Nevertheless, this type of DNA damage has a major role in radiation-induced cell killing [3,4].

Methods previously used for measurement of DSBs, i.e. neutral filter elution, neutral sedimentation or conventional electrophoresis, required very high doses (10^2-10^3 Gy), far above the dose range where cell survival is measurable. The development of pulse-field electrophoresis enabled separation of very high molecular weight double-strand DNA molecules [5,6] and thus, quantification of DSBs resulting from sublethal (<10 Gy, [7]) radiation doses [8-10].

We used a new generation pulse-field electrophoresis system, CHEF-DR III (Bio-Rad). In a preliminary study, we estimated DSBs resulting from 0.1 to 10 Gy of X-radiation and compared the efficiency of DSBs repair in 5 lymphoid cell lines: Reh, human acute lymphoblastoid leukemia (B-lineage); GM00717C and GM00736A, transformed human lymphoblasts derived from *ataxia telangiectasia* patients, homozygous and heterozygous respectively; L5178Y-R (LY-R) and L5178Y-S (LY-S), mouse lymphoma (T-lineage), resistant and sensitive to X-radiation respectively.

The cells were irradiated at 0°C (initial damage) or at 37°C (repair experiments), embedded in agarose and lysed. The resulting nucleoids in agarose plugs were then placed into wells in 1% agarose gel and subjected to electrophoresis in 0.5 x TBE buffer, at pH=8.0, at 6 V/cm, for 24 h, at 14°C. The angle between two current directions was 120° with initial and final switch times 60 s and 120 s respectively. After the electrophoresis, DNA in the gel was stained with ethidium bromide and photographed under UV light (Fig.1). With equal densities of the cells in the agarose plugs, the levels of DSBs were reflected by the quantity of DNA entering the gel and distribution of DNA fragments (Fig.1A). The dose of 2 Gy induced a distinct

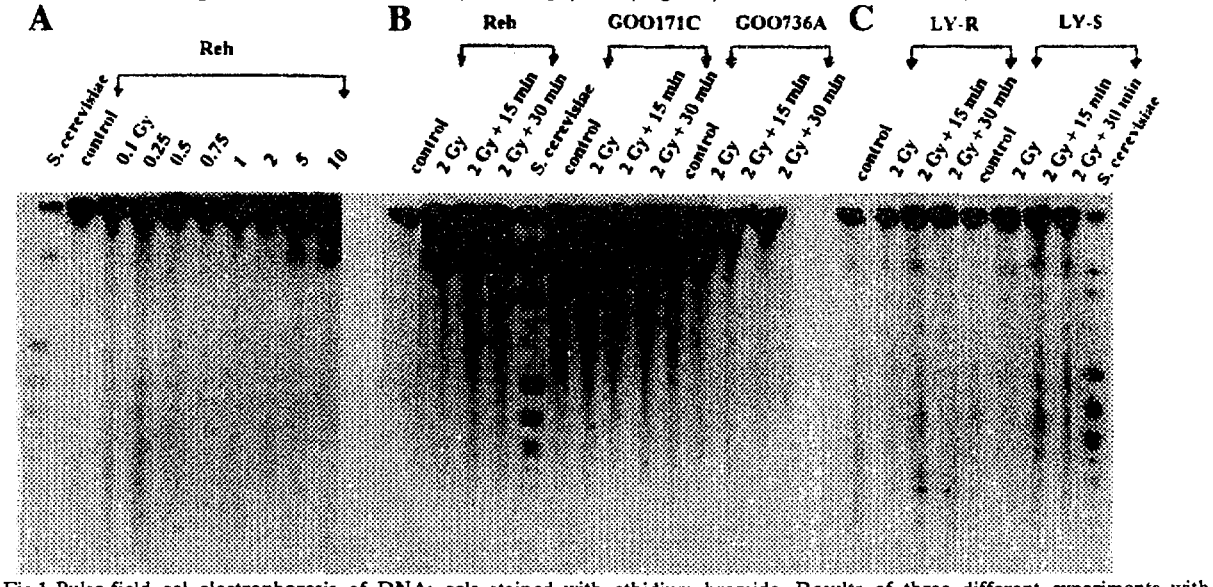

Fig.1. Pulse-field gel electrophoresis of DNA; gels stained with ethidium bromide. Results of three different experiments with X-irradiation are shown: A - DSBs induction in Reh cells irradiated with increasing doses of X-rays at 0°C (ca. $0.5x10^6$ cells/lane); B and C - DSBs repair after 2 Gy of X-rays in 5 lymphoid cell lines (ca. $1.5x10^6$ cells/lane). Control - unirradiated cells; 2 Gy - cells irradiated at 0°C with the dosis of 2 Gy; 2 Gy + 15(30) min - cells irradiated with the dosis of 2 Gy at 37°C and then let to repair the damage for 15(30) min at the same temperature; S.cerevisiae - Saccharomyces cerevisiae artificial chromosomes used as molecular weight markers, range 240-2200 kb.

increase in DSBs over unirradiated control and was chosen for the repair experiments (Fig.1B and C).

Of the cell lines examined, LY-R and GM00736A showed a substantial decrease in the level of DNA damage 30 min after irradiation. In the case of LY-S, GM00717C, and Reh cells no apparent difference was seen between the initial (time "0") DSBs and the DSBs 30 min after irradiation (Fig.1B and C). DSBs repair deficiency in LY-S cells and homozygous *ataxia telangiectasia* cells was in agreement with the previous reports [11,12]. A defect in DSBs repair after X-irradiation in human Reh cell line has, to our knowledge, not yet been described.

The results presented here are a pilot study. To enable studying DSBs repair after biologically relevant doses, sensitivity of the method has to be further improved. This can be achieved by adjusting electrophoretic conditions and enhancing the DNA detection efficiency. There is a possibility to use a new DNA dye with fluorescence much exceeding that of ethidium bromide (e.g. GelStar, FMC Bioproducts, USA) - for direct staining of DNA in the gel, or DNA transfer from the gel to a nylon membrane (Southern blotting) and subsequent identification of the bands with a specific digoxygenine-labelled DNA probe and immunoenzymatic, signal amplificating reaction.

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CELL CYCLE ARRESTS AND APOPTOSIS IN X-IRRADIATED DIFFERENTIALLY RADIOSENSITIVE L5178Y SUBLINES

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We examined the cell cycle arrest, apoptosis and expression of BAX and BCL2 proteins in two L5178Y (LY) murine lymphoma sublines, LY-R and LY-S. They differ in radiation sensitivity slower double strand break (DSB) rejoining is seen in LY-S (radiation sensitive). Both sublines carry a mutation in codon 170 of p53 [1]. LY-R cells do not constitutively express BCL2, whereas both sublines show high BAX content. The levels of these proteins do not change after irradiation. There is no G1 arrest and a difference in G2 arrest, short in LY-R cells and long in LY-S cells. Radiation induces delayed apoptosis to a greater extent in LY-S than in LY-R cells; it can be seen 24 h after irradiation (2 Gy) of LY-S cells, with a maximum at 48 h; LY-R cells need 5 Gy and 72 h to show marked apoptosis (identified by the flow cytometry TUNEL method (Fig.1A) and the "ladder" pattern of DNA electrophoresis (Fig.1B)). The effect revealed by both methods was considerably more pronounced in LY-S cells than in LY-R cells.

In the TUNEL method apoptotic LY-S cells were seen 24 h after irradiation and the maximum was reached at 48 h. The electrophoretic pattern for LY-S irradiated with 5 Gy was closer to a smear than to ladder pattern at 72 h after irradiation (Fig.1B), indicating late necrosis. Apoptotic LY-R cells could be found at later time intervals and

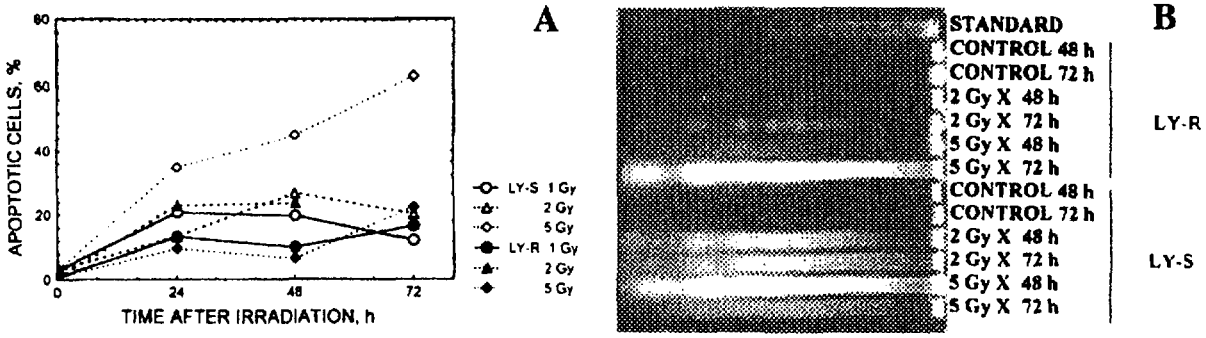

Fig.1. Apoptosis in X-irradiated LY cells. A - % of apoptotic cells determined by flow cytometry-TUNEL in cell cultures X-irradiated with 1, 2 or 5 Gy; B - patterns of DNA electrophoresis (standard - DNA from apoptotic, heat-treated LY-S cells).