INFLUENCE OF X RADIATION ON DAMAGE AND REPAIR CAPACITY OF HUMAN LYMPHOCYTE DNA FROM DONORS OF DIFFERENT AGE

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We have studied influence of X radiation on damage and repair capacity of human lymphocyte DNA from donors of various age employing the micronucleus test and the comet assay. The blood was collected from two groups of donors: "young donors" aged 24–29 years and "elderly donors" aged 58–68 years. Firstly the level of DNA damage was measured on the basis of the micronucleus test. Heparinized blood samples were X radiated (195 kV, 18 mA) in the dose range of 0–3.6 Gy (1 Gy/min). After irradiation the lymphocytes were stimulated with PHA (10 µg/ml). The cytochalasin block was induced by cytochalasin B (5 µg/ml) addition in the 44th hour after stimulation. Fixed and stained microscope slides were analyzed by light microscope. From each culture at last 1000 binucleated cells were examined to record the frequency of micronuclei. To analyze damage and repair capacity by the comet assay, lymphocytes were isolated in a density gradient of Histopaque. Lymphocytes suspended in 0.7% LMP agarose were put on microscope slides precoated with 0.5% NMP agarose. The slides were irradiated with doses of 3, 6 and 9 Gy. For estimation of the repair capacity the slides were incubated in RPMI medium with 10% FBS at 37ºC for 30 min. Electrophoresis was conducted at pH 13. The slides were analyzed in a fluorescence microscope connected with a Lucia-Comet image analysis system ver. 4.51.

The number of spontaneous micronuclei varied from 2 to 10 for "young donors" and from 6 to 12 for "elderly donors". The frequentcy of micronuclei increased with the dose of radiation. The number of micronuclei for "young donors" was even 200 times higher after the dose of 3.6 Gy in comparison to control slides. For "elderly donors" the number of micronuclei increased 34 times. With increasing doses, there was also an increase in the damage to DNA measurement by the comet assay.

Because of significant individual differences in the marked parameters, it is difficult to estimate the differences in radiosensitivity and in the possibility for postirradiation DNA repair between the both groups studied.