BIOPHYSICS AND BIOCHEMISTRY

Comparative Study of Postradiation Genotoxic Changes in Mammalian Cells by Biochemical and Cytogenetic Methods

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The results of studying the genotoxic effect of radiation on endometrial cells of irradiated rats using comet assay correlated with the index of spontaneous DNA degradation. Studying the genotoxic effect of *in vitro* irradiation (2 Gy) on human lymphocytes by means of chromosome analysis and fluorometric assay of nucleoids DNA in irradiated leukocytes also yielded similar results. Measuring of the biochemical marker holds much promise as an express method of evaluation of the genotoxic effect of radiation on mammalian somatic cells.

Key Words: *γ-radiation; comet assay; chromosome aberrations; fluorometry of nucleoid DNA*

Apart from standard methods for evaluation of the genotoxic effects of radiation (counting of chromosome aberrations, study of DNA breaks, and micronucleus test), new approaches are now widely used (comet assay, cytogenetic analysis in combination with fluorescent *in situ* hybridization, evaluation of apoptosis markers, *etc*.) providing detailed characteristics of changes in the content and structure of cell DNA. Study of chromosome aberrations in cultured lymphocytes is a standard method of identification of the effect of radiation on human organism [2,7]. However, this method cannot be used for large-scale studies because its laboriousness and long duration. A more sensitive and rapid method for studying the sufficient number of test objects is required to provide statistically reliable data on the genotoxic effect of radiation. Comet assay is used for rapid evaluation of the genotoxic effect of radiation [7,9]. Study of DNA nucleoids in blood leukocytes with the antibody-specific fluorochrome can be used during the early postirradiation period for rapid diagnostics of radiation injuries in humans and animals [3,6].

Here we compared some standard methods for evaluation of postradiation genotoxic changes in mammalian somatic cells with new methods developed to shorten the time of the study.

MATERIALS AND METHODS

The effect of radiation was studied in 59 female rats obtained from the Rappolovo nursery [1]. The animals were divided into 8 groups. Unirradiated rats served as the control (group 1). Other animals were subjected to whole-body γ-irradiation in single doses of 0.2 Gy (groups 2-7) and 2 Gy (group 8) on an IGUR-1 device (^{137}Cs, dose rate 0.4 Gy/min).

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The rats of groups 2 and 8 received no additional treatment. Group 3 animals received radioprotector N-acetylcysteine (100 mg/kg through a probe, 5 times a week) starting from the 1st day of the study; group 4 rats received 50 mg/kg vitamin C (intraperitoneally) and 40 mg/kg vitamin E (intravenously); group 5 animals received subcutaneous injections of melatonin in a dose of 1 mg/kg; group 6 rats received 100 mg/kg carnosine (intraperitoneally); group 7 animals were forced to swim 5 times a week (the duration of swimming progressively increased from 5 to 60 min).

DNA damage in rat endometrial cells was studied 24 h after irradiation. The efficiency of various methods for correction of radiation damage was evaluated. A modified comet assay is used to study the cells of solid tissues [9]. The comet assay is based on single cell gel electrophoresis and serves for quantitative evaluation of DNA breaks. After lysis and electrophoresis of eukaryotic cells embedded in agarose gel, damaged DNA migrates in electric field toward the anode with the formation of a comet-like structure (comet head consists of undamaged nuclear DNA, while the tail contains damaged DNA). DNA in gel was stained with ethidium bromide. DNA content was measured using a fluorescent microscope. The length of the comet tail reflects the formation of single-stranded DNA breaks. In the present work, we counted 100 cells from each rat. The mean length of the comet tail (MCTL) was calculated per cell [1]. Genotoxic changes in cells were also studied by a modified method based on calculation of the DNA index (DI). DI for endometrial cells from rat uterus was calculated as the ratio of DNA content (μg/ml) to the number of cells per ml suspension (not less than 10^4 cells/ml). The cells were counted in a Goryaev chamber and stained with methylene blue. DNA content was measured with a fluorescent dye 4',6-diamidino-2-phenylindole (DAPI, Serva) in a final concentration of 0.1 μg/ml after lysis of the cell suspension under conditions for nucleoid isolation [3]. Variations in DI are determined by changes in the amount of fluorochrome interacting with DNA in nucleoid formed during lysis of cells in the presence of EDTA, 2 M NaCl, and detergent. The structure of DNA in the nucleoid is similar to its native structure, but lacks complex-forming, protein, and lipid components, which facilitates binding of the dye to the substrate. Changes in the number of dye molecules interacting with nucleoid DNA after ethidium bromide staining is determined by relaxation of the polynucleotide supercoil associated with breaks of pentose phosphate chains after irradiation [4]. The measurements were performed on a Hitachi Model-850 fluorometer at excitation and emission wavelengths of 350 and 450 nm. The ratio of DI for rat uterine cells (pg/cell) before and after 3-h incubation of the sample at 37°C was taken as the index of spontaneous DNA degradation (S_0 index). The relationship between variations in S_0 index and MCTL was evaluated.

In *in vitro* experiments we used blood leukocytes from 12 men (31-63 years) exposed to low-dose irradiation during the Chernobyl accident 11 years before the study. For biochemical tests, 1 ml whole venous blood was 2-fold diluted with Hanks solution and divided into 2 samples. One sample was subjected to γ-irradiation with 2 Gy on a Luch-1 device (^{60}Co, maximum dose 0.45 Gy/min). The irradiated and unirradiated (control) samples were incubated at 37°C for 3 h. The cells were lysed for 3-5 min under conditions for nucleoid separation. Cell DNA content was measured with DAPI using calf thymus DNA as the standard [6]. DNA content per leukocyte was calculated and expressed as DI. The S index was calculated as the ratio between DI for unirradiated and irradiated samples. This index served as a criterion for sensitivity of blood cell DNA to radiation. Biochemical parameters were compared with cytogenetic indexes of lymphocytes from the same donors. The blood sample (1-2 ml) was irradiated with 2 Gy and cultured at 37°C for 48-50 h. Metaphase preparations for the chromosome analysis were obtained routinely [2]. The cytogenetic analysis was performed under a BIMAM light microscope (LOMO, ×900). The frequency of chromosome aberrations and number of exchange aberrations (dicentric and ring chromosomes, acentric fragments) were estimated per 100 cells from each patient.

The results were analyzed by standard methods of correlation and regression analysis and Student's *t* test.

RESULTS

The distribution of endometrial cells by phases of the cell cycle and proliferation index remained practically unchanged in irradiated rats (0.2 Gy) [1]. MCTL in irradiated rats was much higher than in control animals (Fig. 1), which reflects the increase in the degree of DNA damage (*i.e.*, number of polynucleotide chain breaks). Various protective treatments had different effects on these changes. Carnosine administration and swimming most significantly decreased the genotoxic effect. Regression analysis with group values of MCTL and S_0 index revealed the relationship between these parameters: MCTL (μ)=2.70±28.62×S_0 (rel. units), $p<0.05$ (Fig.

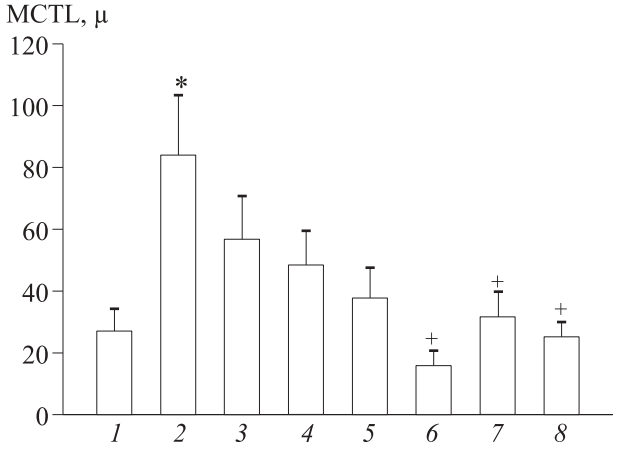

Fig. 1. Effect of irradiation under various conditions on MCTL in rat uterine cells: intact control (*n*=7, *1*); 0.2-Gy irradiation without correction (*n*=7, *2*); 0.2-Gy irradiation+N-acetylcysteine (*n*=7, *3*); 0.2-Gy irradiation+vitamins C and E (*4*); 0.2-Gy irradiation+ melatonin (*n*=7, *5*); 0.2-Gy irradiation+carnosine (*n*=8, *6*); 0.2-Gy irradiation+swimming (*n*=8, *7*); 2-Gy irradiation without correction (*n*=8, *8*). *n*, number of animals. $p<0.05$: *compared to the control; +compared to 0.2-Gy irradiation without correction.

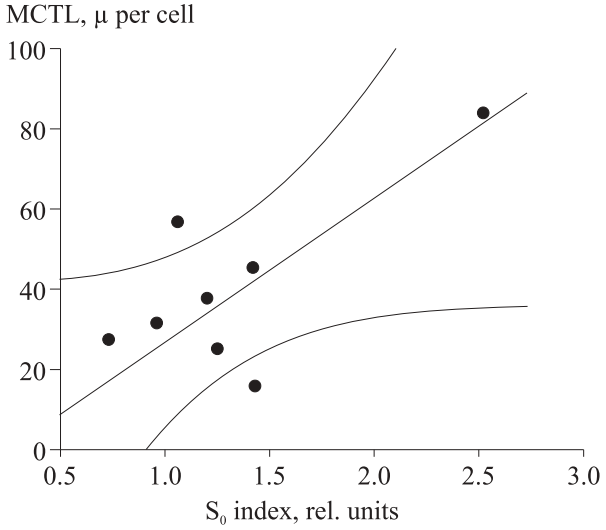

Fig. 2. Relationship between the results of the comet assay and biochemical marker of spontaneous DNA degradation in rat endometrial cells.

2). Similarly to the comet assay, the proposed method of measuring S_0 index in animal somatic cells after irradiation and protective treatment allows evaluation of the degree of DNA damage.

The S_0 index characterizes reparation of DNA breaks in rat endometrial cells after irradiation with various doses. Previous studies showed that the number of double-stranded DNA breaks in cells with different radiosensitivity decreases by 1.8-2 times over 0.5-4 h after X-ray irradiation in a dose of 2 Gy. This effect not observed after irradiation in a dose of 0.2 Gy [8]. It was hypothesized that these changes are associated with the imbalance between DNA reparation enzymes under conditions of irradiation with 2.0 and 0.2 Gy. Our results are consistent with published data. We showed that the number of breaks in endometrial cells decreases to the control level 24 h after irradiation with 2 Gy (as distinct from 0.2 Gy). These changes reflect DNA reparation.

In vitro study showed that interindividual variability of cytogenetic indexes for blood lymphocytes from Chernobyl accident patients reaches 57-76% (frequency of chromosome aberrations) and 30-42% (number of exchange aberrations). Correlation and regression analysis of individual cytogenetic and biochemical markers of radiosensitivity (0.50-1.77 rel. units) revealed a strong positive correlation between variations in the number of exchange aberrations and S index: $N_{dic+ring}(\%)=33.5+5.7\times S$ (rel. units); $R=0.542>R_{95\%}=0.497$ (Fig. 3). The S index did not correlate with the number of acentric fragments and frequency of chromosome aberrations.

Comparative study of individual parameters in 2 age groups of Chernobyl accident patients (31-44 and 45-63 years) showed that evaluation of postradiation genotoxic changes by biochemical and cytogenetic methods yields similar results. No intergroup differences were found in the S index (1.02±0.12 and 1.10±0.21, respectively) and frequency of unstable chromosome aberrations (39.6±2.3 and 40.6±1.4, respectively). These data show that an *in vitro* study of postradiation genotoxic changes in human blood cells by the S index and chromosome analysis of dicentric and ring chromosomes yields similar results.

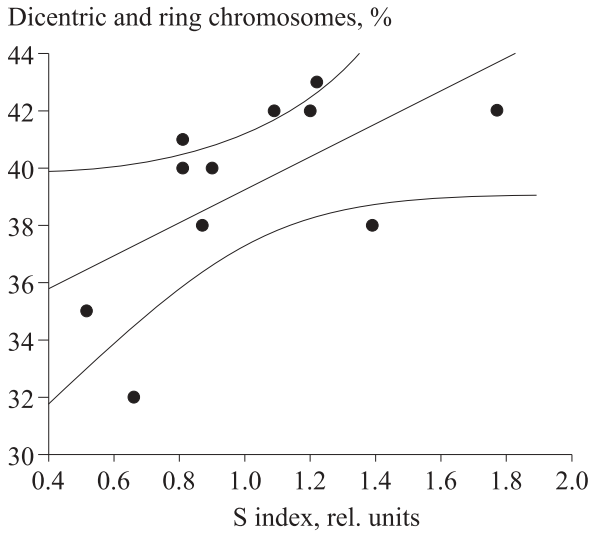

Fig. 3. Relationship between the number of induced unstable chromosome aberrations and biochemical marker for radiosensitivity of DNA in human blood leukocytes.

The increase in the degree of DNA damage in endometrial cells upon exposure to low-dose radiation can be estimated by the comet assay or calculation of the S_0 index (biochemical marker for spontaneous sensitivity of cell DNA). High frequency of unstable chromosome aberrations in human leukocytes exposed *in vitro* to 2-Gy irradiation correlated with the increase in the S index (biochemical marker for induced DNA damage). Both biomarkers allowed us to study DNA damage. It should be emphasized that biochemical markers and number of chromosome aberrations reflected DNA damage in blood leukocytes and mitogen-stimulated lymphocytes, respectively. We conclude that the proposed biochemical markers may be used for evaluation of the genotoxic effect of radiation *in vivo* and *in vitro*. S_0 index can be used in preliminary screening of biologically active compounds preventing the genotoxic effect of low-dose radiation.

Most genotoxic tests for mutagenic or antimutagenic activity of chemical compounds and other treatments take long time. For example, chromosome aberrations are studied for several days. The cells should be incubated for at least 48 h to perform an *in vitro* evaluation of genotoxicity (Ames test with salmonellae/microsomes; micronucleus test with cultures of primary rat hepatocytes or plants) [5]. The time of the comet assay varies from 8 h to 1 day. The S index is calculated over 4 h. The proposed biochemical marker may be used for rapid control of genotoxicity and screening of compounds by biological activity.

REFERENCES

1. L. M. Bershtein, E. V. Tsyrlina, T. E. Poroshina, *et al*., *Byull. Eksp. Biol. Med*., **132**, No. 8, 200-204 (2001).
2. I. E. Vorobtsova, A. V. Semenov, A. Yu. Kanaeva, *et al*., *Radiats. Biol. Radioekol.*, **40**, No. 6, 645-650 (2000).
3. S. D. Ivanov, E. G. Kovan'ko, I. G. Popovich, and M. A. Zabezhinskii, *Ibid*., **39**, No. 4, 418-424 (1999).
4. P. R. Cook and I. A. Brazell, *Eur. J. Biochem*., **84**, 465-477 (1978).
5. C. Helma, P. Eckl, E. Gottmann, *et al*., *Environ. Sci. Technol*., **32**, 1799-1805 (1998).
6. S. Ivanov, L. Korytova, B. Yamshanov, *et al*., *J. Exp. Clin. Cancer Res*., **16**, 413-418 (1997).
7. N. Kopjar and V. Garaj-Vrhovac, *Int. J. Hyg. Environ. Health*, **208**, 179-191 (2005).
8. S. C. Short, S. Bourne, C. Martindale, *et al*., *Radiat. Res*., **164**, 292-302 (2005).
9. S. Tsuda, Y. Kosaka, M. Murakami, *et al*., *Mutat. Res*., **415**, 191-200 (1998).