DNA Reparation and the O_2^--Producing System of Nonspecific Defense of the Organism under Conditions of Induced Radioresistance

V. K. Mazurik, V. F. Mikhailov, L. N. Ushenkova, and N. F. Raeva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 4, pp. 408-411, April, 1997
Original article submitted December 22, 1995

DNA reparation and production of the superoxide anion radical contributing to the genome stability have been studied at different levels of radioresistance. These mechanisms are mobilized under conditions of radioresistance.

Key Words: *genome stability; radioresistance; DNA reparation; superoxide anion radical; somatic mutations*

Stability of the genome is an important factor for preserving the viability of a species under the pressure of genotoxic environmental factors, including ionizing radiation [1]. It is provided by numerous mechanisms, such as DNA reparation [5] and inactivation of defective cells and genetically aggressive bacteria by O_2^--producing blood cells [6]. Genome protection reduces genetic damage and its manifestations, including somatic mutations induced by ionizing radiation, which may cause tumors [8]. Some mutations, for example, occurring in the hypoxanthine-guanine phosphoribosyl transferase locus of human lymphocytes are characterized by the dose—effect quantitative relationship, persist for decades, and are regarded as potential markers of radiation dose and risk of tumorigenesis [11]. In order to investigate the conditions of their appearance, we examined the effect of radioresistance of the organism, an important factor in the formation of any radiobiological effect, on the systems maintaining the genome stability, namely, DNA reparation and production of radical oxygen forms.

MATERIALS AND METHODS

Radioresistance was induced by oral administration of indomethophene (IM, Chemical Pharmaceutical Institute, Russia) in a dose of 30 mg/kg to BALB and outbred mice weighing about 20 g and to dogs weighing 9-20 kg. This dose ensured postexposure survival after lethal irradiation. The IM concentrations adequate to the tissue concentration were created *in vitro*. DNA reparation was studied in murine bone marrow cells exposed to the mutagen N-nitroso-N-methylurea (NMU) and in human peripheral blood cells exposed to ultraviolet (UV) [9]. Mononuclear leukocytes were isolated from the blood of healthy donors on a Ficoll-Urografin gradient [3]. Bone marrow cells were incubated with ^{3}H-thymidine for 1 h, human lymphocytes for 2 h.

The production of oxygen radicals was studied by the NBT test [12] in mononuclear cells isolated from dog peripheral blood [3]. In our model the test permits recording the production of the superoxide anion radical O_2^- [10] stimulated by phagocytosis of latex particles and of other foreign particles and antigens causing a respiratory burst after activation of NADPH dehydrogenase, hexose monophosphate shunt, and increased oxygen consumption [7]. Blood was collected from dogs before, and 4 days 18 h after administration of IM in radioprotectively effective (30 mg/kg) or ineffective (10 mg/kg) doses and 6 h after intravenous injection (immediately after the last blood collection) of 1 mg/kg of lipopolysaccharide pyrogenal in order to mobilize the leukocyte reserve in the peripheral blood [4]. Radioresistance of ani-

State Research Center of the Russian Federation — Institute of Biophysics, Moscow

0007-4888/97/0004-0352$18.00

mals was assessed from the outcome by day 45 after exposure to γ-radiation (^{137}Cs, 7.64 Gy, dose intensity 1 cGy/min). The data were statistically processed as described elsewhere [2], and the results obtained in surviving (radioresistant) and dead (radiosensitive) animals were compared.

RESULTS

The model of IM-induced radioresistance proved to be effective: IM increased the survival of mice from 6.7 to 80% (dose 11.52 Gy) and of dogs from 25 to 93% (7.64 Gy). The synthesis of DNA caused by genotoxic effect of NMU on intact mouse bone marrow cells *in vitro* did not differ from spontaneous DNA production during 3-hour incubation with the mutagen and sharply increased to 275% ($p<0.05$) by the fourth hour, after which it dropped again to the level of spontaneous production (Fig. 1).

The onset of IM-induced radioresistance did not influence spontaneous reparative synthesis of DNA in the bone marrow throughout the observation period. By contrast, reparative DNA synthesis increased to 175% by the third hour of incubation with NMU ($p<0.05$), remained high till the fourth hour, and dropped to the initial values only by the fifth hour. Obviously, the reparation of DNA in bone marrow cells of radioresistant mice starts earlier, i.e., this mechanism of maintaining the genome stability is probably more mobile than in intact animals.

In order to verify this suggestion, we investigated the time course of DNA reparation during exposure of human lymphocytes to UV radiation before and after incubation with IM (Fig. 2). In contrast to intact cells, where UV-induced DNA reparation only after 3 h ($p<0.05$) and normalization was observed only after 4.5 h, cells incubated with IM for 18 h produced DNA as early as after 15 min, their DNA content being 4 times higher ($p<0.01$). Experiments with NMU confirm that the reparative mechanism responsible for the genome stability is mobilized and starts operating earlier in radioresistant than in radiosensitive animals.

The NBT test that reflects the production of O_2^- radical [10] detected slight differences in mononuclear cells isolated from the peripheral blood of spontaneously radioresistant (surviving after exposure) and radiosensitive (dead) animals before exposure during incubation with and without latex particles (Table 1). After the addition of pyrogenal mobilizing the reserve cells in the bloodflow, these changes became statistically significant. It is noteworthy that the decrement (mean difference of individual absolute (neglecting the sign, that is, the trend of changes) values of NBT index in the presence of latex and without it was three times higher in the surviving dogs than in the dead (Table 1).

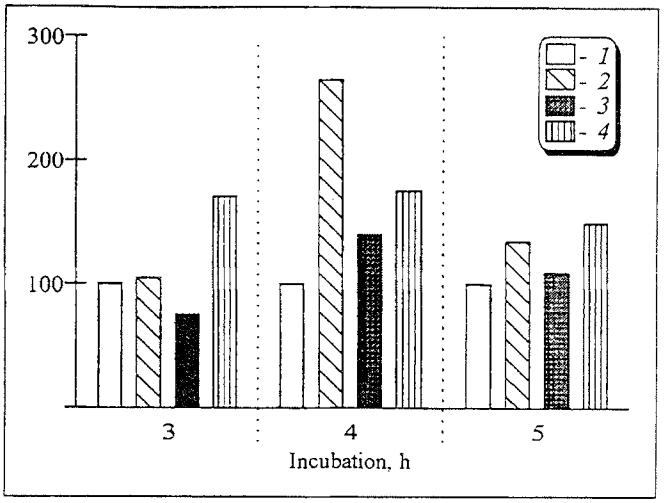

Fig. 1. Spontaneous and N-nitroso-N-methylurea (NMU)-induced reparative production of DNA in mouse bone marrow cells with different radioresistance: natural and induced by 30 mg/kg of indomethophene (IM). Ordinate: reparative synthesis, % of spontaneous DNA synthesis which is taken for 100 for each time interval. *1*) spontaneous production in natural resistance; *2*) NMU-induced synthesis in the same state; *3*) spontaneous synthesis in radioresistance induced by 30 mg/kg of IM; *4*) NMU-induced synthesis in radioresistance after IM in a dose of 30 mg/kg.

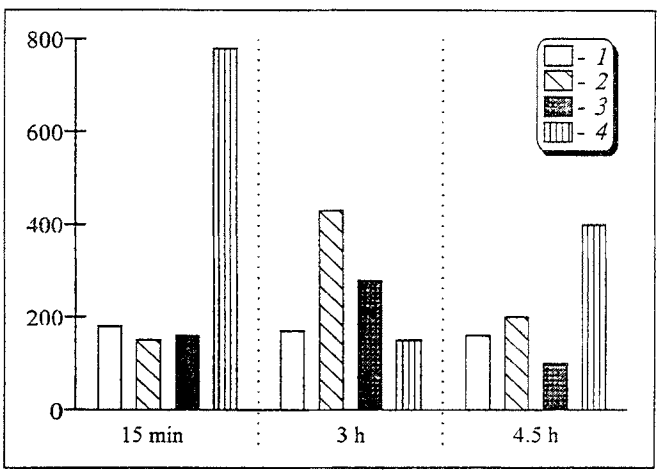

Fig. 2. Spontaneous and UV-induced reparative synthesis of DNA in human blood lymphocytes in radioresistance induced by 18-hour incubation of cells with indomethophene (IM, 30 μg/kg). Abscissa: the beginning of incubation of cells with ^3H-thymidine after UV exposure; ordinate: incorporation of ^3H-thymidine in DNA, cpm/10^6 cells. *1*) spontaneous synthesis in natural radioresistance; *2*) UV-induced synthesis in natural radioresistance; *3*) spontaneous synthesis in induced radioresistance (after 30 mg/kg IM); *4*) UV-induced synthesis in induced radioresistance (after 30 mg/kg IM).

Hence, in case of natural radioresistance another mechanism maintains the genome stability: the superoxide anion radical O_2^- produced by peripheral blood phagocytic cells protects the organism from foreign biological agents.

TABLE 1. The NBT Test with Blood Mononuclear Cells (Optic Units/10^9 Cells) Before and After Pyrogenal Administration to Animals with Natural or Induced Radioresistance

Variants of cell incubation	Type of radiosensitivity or radioresistance			
	natural		IM-induced	
	dead	survived	10 mg/kg	30 mg/kg
Before pyrogenal				
1. With latex	48.6±4.6 (10)	72.4±14.8 (5)	30.0±1.4 (6)	35.7±2.8 (5)
2. Without latex	32.0±6.5 (10)	48.1±5.5 (5)	3.5±1.4 (6)	4.2±0.7 (5)
3. Mean difference of individual values (1-2)	16.6±2.2 (10)	24.3±13.3 (5)	28.7±1.4 (6)	32.9±8.4 (5)
After pyrogenal				
4. With latex	52.9±10.3 (10)	89.9±2.5** (4)	35.7±2.8 (6)	55.3±11.9 (6)
5. Without latex	32.0±6.8 (10)	47.6±4.5* (4)	9.1±2.1 (6)	9.8±2.1 (6)
6. Mean difference of individual values (4-6)	20.9±5.6 (10)	42.2±3.6** (4)	26.6±2.8 (6)	44.8±13.3 (6)
Decrement (3-6)	12.7±5.0 (10)	39.3±9.5* (5)	5.6±1.4 (6)	36.4±7.7** (6)

Note. The number of animals is given in parentheses. Decrement is the mean difference of individual absolute values (3-6). *$p<0.05$, **$p<0.01$ between survived and dead animals.

Similar results were obtained for the decrement using the model of induced radioresistance. The decrement of values before and after pyrogenal did not change in dogs administered IM in an ineffective dose of 10 mg/kg and increased in radioresistant dogs administered IM in a dose of 30 mg/kg ($p<0.01$, Table 1). The biological significance of this phenomenon is unclear. An increase in the O_2^- production inactivates foreign antigens and activates the antioxidant enzymes: superoxide dismutase, glutathione peroxidase, and catalase [7,10], rendering the organism resistant to O-containing radicals and hydroperoxides. Different changes in the latex-induced production of O_2^- in the blood of different species before and after pyrogenal, which increases the decrement (Table 1), indicate that the dogs were at different stages of adaptive changes in the antioxidant defense system. The first stage is characterized by a higher difference in the results of the NBT test with and without latex particles after the addition of pyrogenal, which indicates an increased capacity of cells to produce O_2^-. At stage 2, the index may increase and decrease in the presence of latex (due to induction of antioxidant defense under the action of excessive O_2^- simulating a decrease in the O_2^- production by cells). However, the activity of O_2^--generating mechanisms is high at both stages and is important for the radioresistance of the organism, because it improves antibacterial defense and (at the second stage — adaptation) promotes inactivation of radical oxygen forms generated by radiation.

These data indicate that at least two mechanisms maintaining the genome stability are mobilized in radioresistant cells and organisms: DNA reparation and the system generating oxygen radicals.

The study was supported by the Russian Foundation for Basic Research and the Siberian Trade Bank (Grant No. 95-04-13312a).

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