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Antimutagenic Effect of Adaptation to Stress

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Mutagenesis and, particularly, chromosome mutations play an important role as suppliers of the "raw material for evolution" [3], but at the same time they cause genetic defects found in one tenth of the human population and form the proven or putative basis for thousands of hereditary diseases [15]. This is in conformity with the following data: mutations often arise in the sex cells [2]; in many widespread diseases they are recessive, i.e., do not manifest themselves in the first generation [14]; newly synthesized chemical compounds, which now number in the 3-4 millions, frequently become potential mutagens [11]. Thus, induced mutagenesis harbors multiple risks for the health and lives of the present and future human generations. In response to this threat, antimutagenic influences are now being extensively studied, especially in the context of pharmacological protection of the genome [1,7,21]. In an evaluation of the data on antimutagenic protection the following two facts engage the attention: first, the huge and ever-growing presence of chemical and physical mutagens in the human environment has not restrained progress, population growth, or life-span elongation in highly developed countries. In other words, the actual harm wreaked by mutagenesis is less then its potential harm. Second, among the substances successfully employed for antimutagenic protection an important role is allocated to such factors naturally occurring in the living organism as purine nucleotides [20], vitamins, amino acids, and antioxidants [6]. All these facts suggest that the organism possesses an antimutagenic self-defense system, whose efficiency varies depending on the presence or absence of stable adaptation to a variety of environmental factors. However, the effect of adaptation to particular environmental factors on the resistance of animals and man to mutagens has not yet been studied.

The aim of the present study was to investigate the effect of preliminary adaptation to repeated short-term stress factors or periodic hypoxia on chromosome aberrations in nuclei of the bone marrow stem cells, induced by dioxidine, a well-known chemical mutagen.

MATERIALS AND METHODS

Experiments were performed on C57Bl male mice weighing 18-20 g (Svetlye Gory nursery, Russian

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Academy of Medical Sciences). Five animals were used for each experimental series and overall 6 series were carried out (listed in Table 1).

Adaptation to repeated pain stress consisted of ten stressor influences which were delivered every other day in a cell with an electroded metal floor. The first exposure lasted 5 min, the second 10, and the third and subsequent exposures 20 min, current strength being 0.05, 0.1, and 0.15 mA, respectively. The experimental animals reacted to this electrical pain stimulation by running around and squeaking faintly. In the subsequent exposures these reactions were absent and the mice just occasionally moved from one compartment to another.

Adaptation to hypoxia was performed in a pressure chamber, where hyperbaric hypoxia was gradually produced as follows: the first elevation corresponded to 1000 m, the second to 2000 m, and the third and all subsequent elevations to 3000 m. Each elevation lasted for 4 hours. The elevations were performed every day except Sundays. The total course consisted of 30 sessions of hypoxia.

Analysis of the data obtained in these series enabled us to evaluate the influence of the preliminary adaptation to repeated stress and to periodic hypoxia on dioxidine-induced chromosome aberrations. Dioxidine was administered intraperitoneally in a single dose of 300 mg/kg. The animals were sacrificed 24 h later.

For the analysis of chromosome aberrations in the murine bone marrow stem cells, specimens were prepared according to the Ford and Hamerton method [12], allowing for estimation of the chromosome aberrations in metaphase. For the accumulation of stem cells in metaphase the mice were injected intraperitoneally with 0.025% colchicine (Serva) in a dose of 0.01 ml/g body weight two hours before sacrifice. The animals were killed by cervical dislocation, the femurs were promptly removed, and bone marrow cells were washed out with warm hypotonic solution (0.55% KCl, 37°C, 3 ml per mouse). The cell suspension was incubated at 37°C for 15 min, then pelleted at 1000 rpm for 5 min in an OPN-3 centrifuge. After the supernatant was decanted, the pellet was fixed with 3 ml fixative mixture (ethanol:glacial acetic acid, 3:1) for 10 min, followed by recentrifugation and fixative replacement. Twenty minutes later, the fixative was replaced again. After another centrifugation, the pellet was carefully resuspended in 0.5 ml cooled fixative, and 6-8 drops of the mixture were placed on lipid-free cold slides and dried in the flame of a bunsen burner.

The cytogenetic preparations were stained with azure-eosin for 15 min. The dye contained 0.1% azure:distilled water:0.1% eosin in the ratio 5:10:2, and 5 drops of 5% Na_2CO_3 were added to each 17 ml of the dye.

The cytogenetic analysis was performed after Malashenko *et al.* [5]. In the analysis, single and paired fragments, and chromatid and chromosome exchanges were counted. Achromatic gaps, which according to Gerhard [13] represent the most typical chromosome aberrations induced by chemical mutagens, were specially recorded. However, judging from electron microscopic data, some gaps visible under a light microscope are real chromosome breaks [9]. Therefore, in the present study the mutagenicity was assessed by the total number of cells with gaps and structural chromosome aberrations. One hundred cells from each animal were examined, and each experimental group comprised 5 mice. A Fisher Sci Microscope was used for the cytogenetic analysis.

RESULTS

The main results for different types of chromosome aberrations in animals of the control group and animals adapted to hypoxia and repeated short-term electrical pain stress, which received equal doses of dioxidine, are summarized in Table 1.

As follows from the table, the intact animals (series I) had just a negligible amount of chromosome aberrations: 1.4% bone marrow stem cells contained damaged chromosome. The adaptation either to hypoxia or to repeated stress (series II and III) did not affect reliably the number of chromosome aberrations, and consequently, the adaptation itself does not cause such anomalies.

The data in the table demonstrate that dioxidine was the cause of the chromosome aberrations in 11% of the examined cells, a chromosome fragmentation and the appearance of single fragments (5.6%) being predominant. Multiple chromosome aberrations were detected in 4.4% cells, while such anomalies were absent in intact animals.

The adaptation to hypoxia did not affect these mutagenic effects of dioxidine, whereas the adaptation to stress markedly restricted them. This defense effect was maximally pronounced in respect to single fragments and multiple chromosome aberrations. The number of these two abnormalities was 2-fold lower in mice adapted to repeated stress vs. nonadapted mice, treated with equal doses of dioxidine ($p_{4-6}<0.05$).

The total number of chromosome aberrations in stress-adapted animals was also lower ($p_{4-6}<0.05$).

TABLE 1. Effect of Preliminary Adaptation to Periodic Hypoxia and Short-Term Emotional and Pain Stress on Expression of Dioxidine-Induced Chromosome Aberrations in Bone Marrow Stem Cells

Experimental series		Number of stem cells examined	Different types of chromosome aberrations in % (per 100 cells)					
			gaps	single fragments	double fragments	exchanges	multiple aberrations (>5%)	Total number of chromosome aberrations, %
Control	(1)	500	0.8±0.4	0.6±0.3	–	–	–	1.4±0.5
Adaptation to hypoxia	(2)	400	0.8±0.4	0.8±0.4	–	–	0.3±0.3	1.9±0.7
Adaptation to stress	(3)	400	0.5±0.4	0.5±0.4	–	–	–	1.0±0.5
Dioxidine 300 mg/kg)	(4)	500	0.6±0.3	5.6±1.0	0.2±0.2	0.4	4.4±0.7	11.2±1.4
Adaptation to hypoxia + dioxidine	(5)	400	0.8±0.4	3.3±0.9	–	0.3	6.3±1.2	10.7±1.6
Adaptation to stress + dioxidine	(6)	500	0.6±0.3	2.8±0.7	0.2±0.2	1.0	2.4±0.5	7.0±1.1
Reliability of differences			$p_{4-6}<0.05$		$p_{4-6}<0.05$	$p_{4-6}<0.05$		

Thus, a nondrug factor, adaptation to repeated stress, reliably preserved the chromosome apparatus in the bone marrow stem cells from a heavy dose of chemical mutagen.

In an analysis of the mechanisms of this antimutagenic effect the following three circumstances are to be taken into consideration:

1. The mutagen dioxidine is an inductor of free-radical oxidation [8]. The reactive oxygen forms may be the factor which damages DNA and chromosomes. Adaptation to stress activates the antioxidant systems and may thus restrict the mutagenic effect of dioxidine [17]. However, in a more in-depth analysis such an assumption is highly improbable, since adaptation to periodic hypoxia has now proved to activate considerably the main antioxidant systems in the organism [6] while not exerting any antimutagenic effect (Table 1). It is also important to remember that adaptation to hypoxia is accompanied by activation of the prostaglandin, opioidergic, and GABA-ergic systems, which contribute to the protective effects of adaptation against a variety of damaging influences [17]. The lack of an antimutagenic effect of adaptation to hypoxia essentially indicates that the above regulatory systems cannot protect the chromosome apparatus from such a potent chemical mutagen as dioxidine. Evidently, the antimutagenic effect of adaptation to stress must be determined by a factor which is engaged during adaptation to stress and which is absent in adaptation to hypoxia.

2. An important phenomenon comes to the fore in adaptation to repeated stress, whereas it is just barely in evidence in adaptation to hypoxia. This phenomenon is an enhanced expression of the genes encoding the heat shock proteins (hsp) of aproximately 70 kD. This results in the accumulation of several isoforms of these proteins in the cytoplasm, accompanied by a considerably increased resistance of cell structures to a wide range of damaging factors from ischemia to ionizing radiation [17]. This phenomenon is known in the literature as the phenomenon of adaptational stabilization of structures [18] and is attributed, first, to the disaggregation properties of hsp, which prevent damage-induced protein denaturation, and second, to their ability to block the calmodulin receptors [10,16,22], thus preventing the activation of cell proteases. It is important that in adaptation to stress certain hsp isoforms accumulate in both the cytoplasm and nuclei [18], whereas they are absent in nuclei for adaptation to hypoxia [19]. In line with this, isolated nuclei from animals adapted to stress are highly resistant to single-stranded DNA, an activator of nuclear nucleoproteases [18], whereas in adaptation to hypoxia such resistance was not observed [19]. The data suggest that the antimutagenic protection in adaptation to stress observed by us is due to this nucleoprotective effect of hsp and represents one more manifestation of the phenomenon of adaptational stabilization of structures (PASS). This hypothesis requires verification with other mutagens and a study of gene mutations.

3. When evaluating the biological importance of the established antimutagenic effect of adaptation to stress, we should bear in mind that there are always animals and people nonadapted to hypoxia, cold, heat, physical load, etc., but there is no organism which has never come into contact with a new and hence somewhat stressful situation. In other words, the adaptation to repeated stress is the most common type of adaptation to the environment. In this context it becomes clear that this type of adaptation is most likely to be the factor improving the individual and populational safety of living systems. Moreover, the antimutagenic effect could secure the adaptational protection of not

only the present generation but also the generations to come.

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β-Carotene Stimulation of the Reaction of Cell Immunity in Mice

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β-carotene, or provitamin A, is an effective drug possessing anti-infectious, radioprotective, and, in some cases, antitumor activities [9,15,17,18]. A variety of defense functions of β-carotene have been shown to be related to a great extent to its stimulatory effects on cell, humoral, and antitumor immunity [2,8,19].

The aim of the present study was to investigate the immunomodulatory influence of synthetic β-carotene and its novel nutritional microgranulated form (a Solnechnyi dry milk product, manufactured by the Research Institute of Milk Production, Stavropol') on the reactions of cell immunity in mice, T-cell mitogen-induced lymphocyte prolifera-

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