Hematological and Toxicogenomic Effects of Ferromagnetic Screening of Natural Electromagnetic Fields

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 151, No. 1, pp. 44-47, January, 2011
Original article submitted December 22, 2009

Aftereffects of ferromagnetic screening on the hematological and toxicogenomic parameters in rats were traced over 45 days. Two-day ferromagnetic screening of male and female rats (reducing permanent constituent of magnetic field induction by 4-10 μT) led to leukopenia observed on day 14 of the experiment. Life-time evaluation of the toxicogenomic effects was carried out by rapid method for measurement of blood nucleotide DNA by fluorescent indication. In male blood leukocytes, increased aneuploidy and polyploidy were observed after 48 h of ferromagnetic screening and remained high on days 12 and 28 after screen removal. In contrast to males, leukocyte apoptosis in females was increased only 48 h after the start of ferromagnetic screening.

Key Words: *rats; magnetic fields; ferromagnetic screening; leukopenia; blood nucleotide DNA*

Studies of the effects of environmental factors on humans and animals in places where they spend an appreciable part of their time, for example, in screened constructions, are an important problem of modern ecology. Specific environment with modified natural electromagnetic fields forms inside these constructions, in which the inner environment is partially or completely insulated from the outer environment by ferromagnetic materials. Distortion of electromagnetic fields in the living, industrial, and communal buildings can be caused by elements of ferroconcrete constructions (walls, floor, ceilings) and the ferromagnetic engineering and technological equipment. The electromagnetic fields in general can be characterized by such mutually dependent components as the magnetic field strength and magnetic induction. Experimental studies of magnetic field distribution in living and industrial buildings indicate significant distortions of geomagnetic fields (GMF) in them [9,11].

Studies of the biological effects of reduced and distorted GMP on animals and clinical hygienic studies in special screened constructions showed negative effects of reduced natural magnetic field on the CNS, cardiovascular and immune status, and on the status of other organs and systems [1,3,7,9,10]. Possible negative effects of GMF fluctuations on embryogenesis and early ontogenesis, eventually leading to the development of unfavorable delayed effects, were studied [2,13]. However, no data on possible genetic effects on mammals enclosed in ferromagnetic-screened (FS) constructions have been reported. We studied possible aftereffects of temporary FS on changes in GMF level and toxicogenomic parameters of rats.

MATERIALS AND METHODS

Three series of experiments on outbred albino rats (12 experimental and 12 control males and females; 180-200 g) were carried out. Experimental rats were screened by a U-shaped steel screen (0.1 cm thick),

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0007-4888/11/1511-0038

30×30×30×55 cm, covering the standard cage from two sides and forming the roof. Controls were screened by carton screens of the same size and shape. Drinking water and fodder were given from the end side of the cage according to a common schedule. The animals were kept under these conditions for 48 h, after which the screens were removed and the animals were observed during a period of up to 45 days.

Measurements of induction of permanent magnetic field constituents inside the cages with rats were carried out with a Neva-4 teslameter. The levels of GMP disturbances on the days of the experiment were evaluated at Geophysical Laboratory of St. Petersburg Affiliated Department of Institute of the Earth Magnetism and Ionosphere and Radiowave Propagation by the common criteria and were expressed as Ki indexes.

Hematological and toxicogenomic parameters were evaluated in living animals as follows. The blood (0.05 ml) was collected from the caudal vein 5, 24, and 48 h after the start of FS and 12, 28 days and 1.5 months after the screens were removed.

Blood leukocytes were counted by the melanger chamber method, differential leukocyte count was determined by microscopy of stained smears on slides.

Toxicogenomic effects in rat leukocytes were evaluated by an express method: DNA index (DI; nucleotide DNA content per leukocyte) was determined using fluorochrome 4',6-diamidino-2-phenylindole as described previously [5]. Fluorescence was measured on a Model-850 fluorescent spectrophotometer (Hitachi) at stimulation λ=350 nm and emission λ=450 nm. DI for peripheral blood leukocytes from intact rats (rel. units per cell) corresponded to diploid (2c) DNA content in white blood cells. If the DI value was below 2c, the count of cells with DNA content below the diploid was assumed to be increased at the expense of apoptotic death of part of DNA-containing blood cells. These changes in DI values were verified by parallel analysis of the same samples by flow cytometry [4].

The increase in DI above 2c was caused by an increase in the count of cells with hyperaneuploid and tetraploid DNA contents, which was found by comparing these data with the results of cytogenetic analysis of the same samples in special experiments [6]. Low-dose mercury and radiation exposure served as the reference destructive exposure in these experiments. The animals received $Hg_2(NO_3)_2$ in a concentration of 1 μg/liter in conversion to mercury (maximum allowable concentration) with drinking water for 30 days before and 30 days after single X-ray exposure in a dose of 25 cGy. After this, DI and number of chromosome aberrations were evaluated by the standard method [15]. The level of hyperaneuploid and polyploid lymphocytes in intact rats was 1.0-1.1% and after radiation and mercury exposure 2.0-2.2%. The corresponding DI values were 8.0-8.7 rel. units/cell for the control and 14.8-16.0 rel. units/cell after radiation and mercury exposure. Regression analysis of individual levels of hyperaneuploid and polyploid lymphocytes (in %) and the corresponding blood leukocyte DI values in rats showed a significant (R=0.99; p<0.01) relationship between these parameters:

$$\text{hyperaneuploidy and polyploidy (\%)} = 0.1+0.14\times\text{DI (rel. units per cell)} \quad \textbf{(1)}.$$

Hence, using the above method for DI evaluation in rat blood leukocytes after radiation and mercury exposure, we evaluated the increase in the levels of leukocytes dying by apoptosis and of hyperaneuploid and polyploid lymphocytes.

The majority of genotoxic tests used for biotesting of destructive exposure of different kinds take rather much time for evaluation of the needed parameters. DI is determined in just 0.03 ml blood within 10-15 min. Hence, rapid evaluation of this parameter can be used for express evaluation of toxicogenomic effects of physicochemical exposure in somatic cells of mammals and small laboratory animals.

RESULTS

The external levels of GMF distortions evaluated during the periods of animal screening were rather stable: 13.0±3.6 Ki indexes/day for males and 17.3±4.2 Ki indexes/day for females.

Measurements of permanent magnetic field induction showed magnetic field of 53.3-63.5 μT in the zone with control rats. Permanent magnetic field induction in the zone of the cage with animals screened by steel was 49.4-52.3 μT, that is, 3.9-10.2 μT lower than in the zone with controls.

Over 1.5-month follow-up, blood leukocyte counts in experimental animals decreased only on day 12 after the steel screen removal: 70.7±2.3% in males and 66.4±5.2% in females in comparison with the controls (p<0.05). Analysis of differential blood count showed that changes in the total count of white blood cells in experimental males were mainly due to lymphocytes (their percentage decreased to 68.0±5.8% compared to the control, p<0.05). The count of other white blood cell fractions virtually did not change in males over 1.5 months. In females, no appreciable changes in the counts of any of white blood cell fractions were noted over the entire period of observation.

Leukocyte DI in male rats gradually increased over 48 h, reaching 155% of the control (Fig. 1). After FS, DI slightly increased to 165% of control by day 14; by the end of month 1 after the beginning of the experiment it was 155%, and by the end of observa-

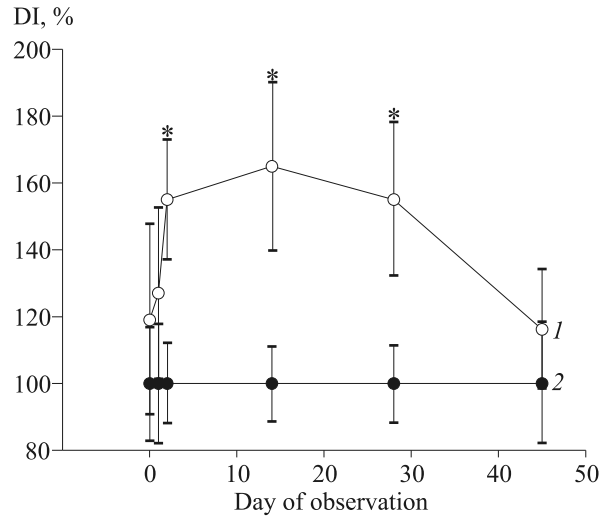

Fig. 1. Changes in blood DI in male rats during the experiment. *1*) experimental group; *2*) control group. *$p<0.05$ compared to the control.

tion (1.5 months) it decreased to the control level. DI in intact rats and equation **(1)** suggest that additional 0.5% of hyperaneu- and polyploid lymphocytes appeared in the blood of experimental males during this period. It was previously shown that low-dose radiation and mercury exposure led to an increase in blood DI [12] and later to a 38-40% increase in the incidence of tumor formation in experimental compared to control rats.

A decrease of DI below 2c (to 70±8% *vs.* control, $p<0.05$) was found in the peripheral blood of females. In other words, the count of apoptotic leukocytes increased only after 48 h of FS. During the subsequent 45 days the blood DI of experimental females did not differ from the control.

Hence, 48-h FS of rats led to lasting elevation of the lymphocyte hyperaneu- and polyploidy on days 2-30 in the males and a transitory increase of apoptotic leukocytes on day 2 of the experiment in females.

Our results are in good agreement with published reports [1,3,7] on unfavorable effects of hypogeomagnetic field on males and on possible normalization of the detected shifts. The aggressiveness of male rats increased and plasma antiradical activity decreased in animals exposed to hypogeomagnetic field (~100 nT) for 10 days [8]. These biochemical changes in the blood were in line with the increase in leukocyte DNA ploidy in male rats in our study. In contrast, in females estradiol stimulated antiradical processes by increasing the expression of intracellular antioxidant enzymes [14], this preventing the development of hyperaneu- and polyploidy, which was observed in our experiments.

A permissible level of GMF reduction at working places in screened constructions and in buildings with many metal (iron-containing) elements has been determined in the Russian Federation. On the other hand, metal constructions are built and the houses are faced by steel panels without due biomedical validation up to the present time. Our findings indicate that FS involving attenuation of magnetic fields by even just 4-10 μT during 48 h leads to significant, though transitory hematological and toxicogenomic aftereffects in the rats, mainly males, which can result in the development of delayed unfavorable consequences. The regularities found in our experiments are just preliminary data. However, wide use of metal materials and steel constructions in designing, building, reconstruction of living and communal buildings necessitates studies of FS effects on human health.

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