Effect of Magnetic Fields on Antioxidant System Enzymes in Mice with Ehrlich Ascites Carcinoma

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The combination of weak steady-state and weak low-frequency alternating magnetic fields activates SOD in Ehrlich ascites carcinoma cells and catalase in liver cells by 3.7 and 1.3 times, respectively ($p<0.05$), which can result from enhanced production of ROS induced by combined exposure to magnetic fields with the specified parameters.

Key Words: *magnetic fields; catalase; superoxide dismutase; Ehrlich ascites carcinoma*

In recent years, the problem of tumor diseases aggravated. The researchers focus on a number of physical factors that can affect tumor growth. Among such factors, magnetic fields are most interesting. Experimental biology and clinical practice accumulated numerous data on efficiency of magnetic therapy. Specifically, magnetic fields affect the sympathoadrenal system, motor activity of myocytes, glutathione level, and higher nervous activity [7,10,12]. A multitude of hypotheses on this problem attests to its disputable character rather than to adequate general conception of how natural and instrumental magnetic fields affect the living creatures [1].

The study of living organism with the methods of quantum mechanics showed that *in vivo* chemical reactions have much in common with the test-tube reactions indicating that the mechanism of magnetic field effects on the living body can be explained by changes in the energy of chemical bonds in biological processes. As a rule, chemical reactions transform the molecules by rearrangement of their electron shells of the atoms. The effects of magnetic fields relate to modulation of probability of elementary chemical acts at the stage when free-radical products with uncompensated spins appear due to generation of unpaired electrons. The transitions between various spin states of an electron pair are affected by external magnetic field, which thereby changes the probability of the corresponding chemical reactions and produces magnetobiological effects [1].

Our aim was to examine the effect of combined action of weak steady-state and low-frequency alternating magnetic fields on activity of antioxidant enzymes SOD, catalase, glutathione peroxidase, and glutathione S-transferase (GST) in mice with Ehrlich ascites carcinoma (EAC).

MATERIALS AND METHODS

The study was carried out in random-bred albino mice weighing 18-20 g. The mice were inoculated intraperitoneally with 10^7 EAC cells suspended in 0.2 ml Hanks solution. The animals were randomized into control and experimental groups. The experimental mice were exposed to magnetic field for 5 days (1-h daily exposure).

Magnetic field was generated by an original setup composed of a signal generator, control amplifier with graduation of the alternating magnetic field component, a source of stabilized constant current, and an integration block mixing the constant magnetic field (25 μT) with alternating magnetic field (5 μT, 3.12 Hz).

Activity of antioxidant enzymes was determined in ascites cells and in homogenates of the liver and

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bone marrow. The liver and bone marrow were isolated and homogenized in a glass mortar with an electrical homogenizer equipped with a Teflon pestle at 3000 rpm. NaCl (0.9%) was used as the homogenization medium. The liver and bone marrow homogenates were dissolved by 4 and 67 times, respectively. The tumor cells were separated from ascites fluid by centrifuging at 3000 rpm for 15 min, thereafter they were homogenized under the specified conditions. The tumor cells homogenate was dissolved 4-fold.

SOD activity was assessed by the degree of inhibition of epinephrine self-oxidation in alkaline medium containing the enzyme [3]. The intensity of epinephrine self-oxidation was established by the dynamic rise of light absorption at λ=347 nm resulted from accumulation of oxidative products advancing production of adrenochrome with the absorption maximum at 480 nm.

The method to assay catalase activity is based on the formation of yellow-colored complex not destructed during the catalase-catalized reaction of hydrogen peroxide with ammonium molybdate, whose staining intensity was measured on a photoelectric colorimeter at λ=400 nm [2].

Glutathione peroxidase activity was assessed by the rate of oxidation of reduced glutathione (GSH) in the presence of tert-butyl hydroperoxide [5]. The concentration of GSH before and after incubation was determined on a SF-26 spectrophotometer at λ=412 nm. The development of color reaction is based on interaction between SH-groups of reduced glutathione with 5,5'-dithio-(bis)-nitrobenzoic acid (DTNB) resulting in the formation of a colored product, thionitrophenyl anion. The quantity of these anions is directly proportional to the number of SH-groups that reacted with DTNB.

The method to determine GST activity is based on the rate of formation of the glutathione-S-conjugates between GSH and 1-chloro-2,4-dinitrobenzene. Accumulation of conjugates was recorded spectrophotometrically at λ=340 nm [5].

The data were processed statistically by nonparametric Mann–Whitney *U* test using Statistica 6.0 software.

RESULTS

Combined exposure to steady-state and alternating magnetic fields increased SOD activity in EAC cells by 3.7 times ($p<0.05$), but produced no significant effect on activity of this enzyme in liver and bone marrow cells (Table 1).

Catalase activity was measured only in liver homogenate of tumor-carrier mice. The magnetic field with specified parameters significantly increased activity of this enzyme from the control level of $9.00\pm0.86\times10^3$ mcat/g protein to $11.40\pm0.80\times10^3$ mcat/g protein or by 1.3 times ($p<0.05$, $n=15$).

The effect of magnetic fields with the specified parameters significantly increased activity of glutathione peroxidase in the liver and bone marrow by 2.3 and 1.5 times, respectively ($p<0.05$, Table 2). In contrast, activity of this enzyme did not significantly change in ascites cells.

Exposure to magnetic fields markedly increased GSH activity in hepatocytes and in ascites cells by 3.1 and 1.7 times, respectively (Table 3).

Thus, combined exposure to weak steady-state and weak low-frequency alternating magnetic field increased activity of antioxidant system enzymes in EAC, bone marrow, and liver cells of tumor-bearing mice, which can indicate up-regulation of free radical

TABLE 1. Effect of Combined Exposure to Weak Steady-State and Weak Alternating Low-Frequency Magnetic Fields on SOD Activity in Homogenates of Ascites, Liver, and Bone Marrow Cells of Mice with EAC ($n=15$)

Group	Hepatocytes, arb. unit/g tissue	Bone marrow cells, arb. unit/g tissue	Ascites cells, arb. unit×10^{-5}/cell
Control	182.00±1.63	154.29±9.76	0.120±0.028
Experimental	183.40±1.79	155.31±8.16	0.440±0.017

TABLE 2. Effect of Combined Exposure to Weak Steady-State and Weak Alternating Low-Frequency Magnetic Fields on Glutathione Peroxidase Activity in Homogenates of Ascites, Liver, and Bone Marrow Cells of Mice with EAC ($n=15$)

Group	Hepatocytes, μmol/min×g protein	Bone marrow cells, μmol/min×g protein	Ascites cells, μmol/min×g protein
Control	63.3±0.16	61.0±0.34	54.3±0.35
Experimental	144.40±0.80	90.7±0.54	60.4±0.14

TABLE 3. Effect of Exposure to Weak Steady-State and Weak Alternating Low-Frequency Magnetic Fields on GSH Activity in Homogenates of Ascites and Liver Cells of Mice with EAC (n=15)

Group	Hepatocytes, μmol/min× g protein	Ascites cells, μmol/min× g protein
Control	61.2±0.66	36.6±0.12
Experimental	190.5±0.77	61.3±0.47

production under the action of magnetic fields with the specified parameters.

Reactive oxygen species (ROS) are known to induce cell death. Some agents such as motexafin gadolinium that elevate ROS production provoke apoptosis in tumor cells [6]. Similarly, apoptosis of cardiomyocytes was triggered by up-regulating ROS production during traumatic stimulation [8]. ROS can provoke apoptosis via different pathways. There are data demonstrating that ROS can activate caspase cascade via Bcl-2 down-regulation [11]. It is an established fact that moderate oxidative stress can provoke apoptosis, while severe stress induces necrotic cell death [4,13]. Thus, elevation of activity of antioxidant system enzymes in our study attests to up-regulation of ROS production under the action of magnetic fields, which in its turn can trigger either pathway of cell death.

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