EFFECTS OF ENVIRONMENTAL ELF MAGNETIC FIELDS ON MYELOPEROXIDASE (MPO) ACTIVITY

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Abstract : Myeloperoxidase (MPO) is one of the enzymes that constitute the defense system of immune cells. It has an important function in inflammation. In this study, it was investigated the effects of 50 Hz, 10 Gauss (G), 20 G and 30 G magnetic fields on MPO activity in renal tissues of guinea pigs. The experiments were carried out on 50 male, 250-300 g weighted (10-12 weeks aged) guinea pigs. The subjects were divided into one control group (n=8) and six experimental groups (n=7) which were exposed to 50 Hz magnetic fields of 10 G, 20 G and 30 G with the exposure periods of 4 hours /day and 8 hours/day for 5 days. MPO activities in renal tissues of exposed and unexposed guinea pigs were determined by measuring the H_2O_2-dependent oxidation of o-dianisidin. Renal MPO activities were also found increased for 10 G, 20 G and 30 G of the magnetic fields and both of the exposure periods. The increases in MPO activities were found statistically significant under the effect of 10 G (p= 0.004) for the exposure period of 4 hours/day, and 20 G for both exposure periods of 4 hours (p= 0.014) and 8 hours (p= 0.001) respectively.

Introduction

Various cellular components, processes, and systems can be affected by EMF exposure. Since it is unlikely that EMF can induce DNA damage directly, most studies have examined EMF effects on the cell membrane level, general and specific gene expression, and signal transduction pathways. In addition, a large number of studies have been performed regarding cell proliferation, cell cycle regulation, cell differentiation, metabolism, and various physiological characteristics of cells. Although 50/60 Hz EMF do not directly lead to genotoxic effects, it is possible that certain cellular processes altered by exposure to EMF indirectly affect the structure of DNA causing strand breaks and other chromosomal aberrations [1].

Magnetic fields (MFs) influence the kinetics of reactions with radical pair intermediates [2-3]. External MF can increase the concentration of free radicals in living cells. Transition metals, e.g. iron or copper, are among the most important agents that can cause damage of DNA, RNA and other macromolecules through the production of oxygen free radicals (ROS), by Fenton reactions or by interaction with cellular thiols [4]. When ROS react with nonradicals, new free radicals can be formed, which leads to chain reactions, i.e. lipid peroxidation. Zmys´lony et.al. demonstrated that 50 G static magnetic field and iron ions increased lipid peroxidation in isolated rat liver microsomes [5].

Free radicals are intermediates in natural processes like mitochondrial metabolism and are also a key feature of phagocytosis [1]. Macrophages play an essential role in the body's defense and immune system. Activated macrophages release free radicals as reactive oxygen species (ROS), reactive nitrogen species (RNS), and also cytokines. ROS are unstable reactive molecules which are produced continuously in several cells [6]. High-level production of free radicals in the organism has shown an increased potential for cellular damage of substances such as DNA, proteins, and lipid-containing structures [7]. In contrast to molecules such as cytokines (large molecules signaling by docking with specific receptors and change molecular surfaces on the target cells) molecules such as ROS could react with diverse cell compounds in a non-specific mechanism. Therefore, free radicals play a decisive role in cytotoxicity and also as cellular messengers to control non-cytotoxic physiological responses.

In the light of the epidemiologic results, it is worthwhile to review the current state knowledge regarding the effects of extremely low-frequency (ELF) EMF signals on the immune system. Because the immune system functions as the body's main protective mechanism against tumor formation and growth, EMF-induced changes in immune cell biochemistry could affect the organism's immune response directly in either a negative or positive manner. The physiological significance of the epidemiological results as well as of the reported immunological EMF effects, however, cannot be fully evaluated until 1) there are convincing results from whole organism exposure studies that can be directly related to the in vitro evidence, and 2) the biological mechanisms by which weak EMFs may interact with cells of the immune system begin to be understood. With regard to in vivo exposure effects on the organism's immune system, it has already been demonstrated in several independent laboratories that nonthermal ELF EMF intensities can modify blood

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leukocyte counts [8-9], inflammatory responses [10-11], peripheral blood natural killer (NK) cell activity [12], and the appearance or weight of primary and secondary lymphoid organs [12-13].

Myeloperoxidase (MPO) is a bactericidal enzyme secreted by activated phagocytes that constitute the defense system of immune cells, specifically catalyzes the production of hypochlorous acid (HOCl) from chloride and hydrogen peroxide [14-15]

The aim of the present study was to evaluate the influences of in vivo exposures to 10 G, 20 G and 30 G MFs on MPO activity in renal tissues of guinea pigs.

Materials and Methods

Animals

The experiments were carried out on 50 male, 250-300 g weighted (10-12 weeks aged) guinea pigs. The guinea pigs were kept in plastic cages, 26 x 22 x 10 cm, in the laboratory at a room temperature of 23°C, a day and night cycle of 12 hours and ambient geomagnetic field of 0.3 Gauss.

The subjects were divided into one control group (n=8) and six experimental groups (n=7) which were exposed to 50 Hz magnetic fields of 10 G, 20 G and 30 G with the exposure periods of 4 hours /day and 8 hours/day for 5 days.

Experimental Model

To generate magnetic fields, circular coils pair of Helmholtz configuration was used. The magnetic field generated by the coils was classified as vertical field (field lines perpendicular to the bottom plane of the animal's cage). Pair of circular coils of either 42.75 cm diameter and 21.375 cm clearance was constructed by insulated copper wire and made of 154 turns. The electrical parameters of each coil were resistance, 1.2 ohms (Ω); and inductance, 19.6 milliHenry (mH). Sinusoidal current of frequency 50 Hz was generated at the output of the circuit which was droven by the specially designed variable transformer, 2.7 kVA in power. Magnetic field was measured with a Hall-Effect Gaussmeter. Frequency and waveform of the magnetic field were monitored over an ossiloscope.

Guinea pigs were placed in the centre of the Helmholtz coils, 2 per plastic cage and were exposed to 50 Hz magnetic fields with the intensities of 10 G, 20 G and 30 G with the exposure periods of 4 hours/day and 8 hours/day for 5 days. Eight subjects were kept in an identical laboratory conditions without being exposed to any magnetic field and studied as control.

To control possible variation in responses due to the circadian rhythm, exposure periods were chosen between 8:00 - 12:00 a.m. and 8:00 a.m. - 4:00 p.m. respectively.

On the following day after exposure, all of the animals were sacrificed by either inhalation in a closed box. Their kidneys were dissected out immediately. They shocked by liquid nitrogen and stored in deepfreeze at -70°C until performing the analysis of MPO activity.

All experiments were run blind; i.e, the experimenters performing the MPO activity assay did not know the exposure conditions of the animals.

Renal Tissue MPO Activity Assay

Renal tissue MPO activity was determined by the method of Lopez-Neblina et al.[16]. Kidney tissue was homogenized in 20 mM potassium phosphate buffer (pH:7.4) and the homogenate was centrifuged for 5 min. at 10 000xg at 4°C . The supernatant was discarded, and the pellet was resuspended in 50 mM potassium phosphate buffer (pH:6.0) containing 0.5% hexadecyltrimethylammonium bromide (HETAB) and 0,146 % of EDTA. The homogenate was frozen and they once; it was sonicated for 10 seconds, incubated for 2 hour in a water bath (60°C), and then centrifuged at 12 500xg at 4 °C for 30 minutes. The supernatants were used MPO assay.

MPO activity was assessed by measuring the H_2O_2-dependent oxidation of o-dianisidin. One unit (U) of enzyme activity was defined as the amount of MPO present that caused a change in absorbance of 1.0/min at 410 nm. and 37 °C. [17]

Statistical Analysis

Mann Whitney-U test was applied for statistical analysis. Statistical analyses were carried out using SPSS software (SPSS Inc., Chicago, USA). The P value was considered significant at $P<0.05$.

Results

MPO activity was investigated in renal tissues of guinea pigs in the presence of 50 Hz, 10 G, 20 G and 30 G magnetic fields with the exposure periods of 4 hours/day and 8 hours/day for 5 days.

All of the MPO values are mean±sem, standart error of mean. MPO activity in control group was found 0.636 ± 0.085 U / g tissue. For the guinea pigs exposed to 10 G, MPO values in kidneys were found 0.928 ± 0.023 U /g tissue and 0.815 ± 0.045 U /g tissue for the exposure periods of 4 hours ve 8 hours respectively. MPO activities were found 0.914 ± 0.045 U /g tissue for the exposure period of 4 hours and 1.054 ± 0.051 U /g tissue for the exposure period of 8 hours with 20 G exposure. Kidney MPO activities were found 0.876 ± 0.050 U /g tissue and 0.927 ± 0.088 U /g tissue in the presence of 30 G MF with the exposure periods of 4 hours and 8 hours respectively.

Renal MPO activities were found increased for 10 G, 20 G and 30 G MFs in both of the exposure periods with respect to controls. The increases in MPO activities were found statistically significant for 10 G with 4 hours/day ($p= 0.004$) and for 20 G with 4 hours/day ($p= 0.014$) and 8 hours/day ($p= 0.001$). The exposure period of 8 hours/day was found more effective ($p=0.038$). The results are given in Figure 1.

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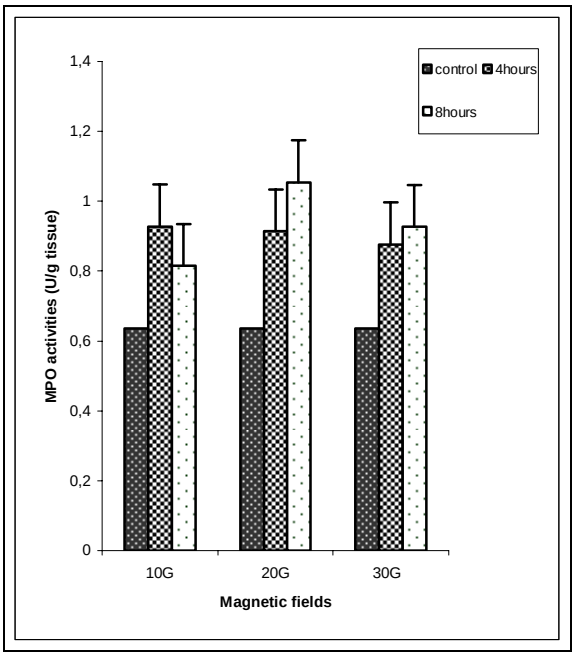

Figure 1. MPO activities of kidney tissues of MF exposed and control guinea pigs

Discussion

Epidemiological studies have shown an association between occupational exposure to extremely low frequency (ELF; <300 Hz) magnetic fields (MF) and certain cancers, such as leukemia and cancer of the nervous system (mostly brain tumors) [18-20]. ELF magnetic fields have been classified as a "possible human carcinogen" by The International Agency for Research on Cancer-IARC [21-23]. ELF Magnetic (B) fields effects on different tissues of guinea pigs under laboratory conditions were carried out at the Bioelectromagnetic Laboratory of Biophysics Department in Medical Faculty of Gazi University.

Simko and Mattsson [1] envisage that EMF exposure can cause both acute and chronic effects that are mediated by increased free radical levels: 1- Direct activation of, for example macrophages (or other cells) by short-term exposure to EMF leads to phagocytosis (or other cell specific responses) and consequently, free radical production. This pathway may be utilized to positively influence certain aspects of the immune response, and could be useful for specific therapeutic applications. 2- EMF-induced macrophage (cell) activation includes direct stimulation of free radical production. 3- An increase in the lifetime of free radicals by EMF leads to persistently elevated free radical concentrations. In general, reactions in which radicals are involved become more frequent, increasing the possibility of DNA damage. Data by Singh and Lai [24-25] support the hypothesis that exposure to a power frequency (60 Hz) magnetic field at flux densities of 1 G, 2.5 G and 5 G cause DNA damage in the cells (rat brain cells) with the involvement of oxygen free radicals processes. Similarly, exposure to 70 G magnetic field, static or 50 Hz, can induce DNA damage in rat lymphocytes if the cells were simultaneously treated with $FeCl_2$.

Free radicals or oxygen species are thought to contribute to the pathology of many diseases. These include inflamatory conditions, where neutrophils accumulate in large numbers and are stimulated to produce superoxide and other reactive oxidants. [26]. The generation of reactive oxygen species (ROS) including superoxide ($O\cdot_2^-$) by the plasma membrane NADPH oxidase of neutrophils is a major mechanism of bacterial killing [27]. The $O\cdot_2^-$ thus formed enzymatically dismutates to hydrogen peroxide (H_2O_2) through catalysis by superoxide dismutase, or is converted to other reactive oxygen intermediates such as hypochlorite by the myeloperoxidase reaction [28].

Myeloperoxidase (MPO) is an enzyme localized in the azurophilic granules of neutrophils. It oxidizes chloride ions to the strong bactericidal oxidant, hypochlorous acid (HOCl), and produces oxygen radicals [29]. It has been shown that phagocyte-generated oxidants are involved in the pathogenesis of cancer by causing DNA strand breaks, activating procarcinogens to genotoxic intermediates, and by inhibition of DNA repair [30-31]. According to all of these information, we have studied MPO activity as an indicator of immune system activity in kidney. Because it has high conductivity, kidney is one of the tissues which is effected most from EM fields.

Numerous studies have addressed the interaction between EMF and calcium fluxes, because calcium is the principal regulator of several cellular processes. Modulations in intracellular Ca^{2+} concentrations during exposure to EMF were reported by various investigators [32-35]. It has been concluded that modulation of intracellular Ca^{2+} concentrations is possible only in stimulated immune cells [36]. Immune system activity under 50 Hz magnetic field was investigated in our laboratory on guinea pigs and found that 20 G magnetic field exposure decreased the NK cell cytotoxic activity [37]. It has been investigated whether electrolyte concentrations of brain and plasma tissues are influenced by ELF magnetic field. It was found that 50 Hz, 20 G magnetic field increased Ca^{2+} concentration in brain and plasma tissues. The increase in Ca^{2+} concentration in plasma was statistically significant [38].

There have been a few published results on effects of time-varying magnetic fields on NK cell activity. NK cell activity under magnetic field was investigated on guinea pigs in the presence of 50 Hz, 20 G Magnetic Field with the period of 4 hours/day for 5 days in our laboratory. It was found that magnetic field exposure decreases the NK cell cytotoxic activity [37]. In the present study, it was observed that 10 G, 20 G, 30 G fields which has been applied for 4 and 8 hours per day, caused an increase in kidney tissue MPO activity. In both application periods, 20 G magnetic field caused statistically significant increase and this

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is consistent with the increase in NK with the effect of 20 G MF.

Greater neutrophil influx into the peritoneum, indicated by higher myeloperoxidase levels, was also observed in NK cell-depleted mice [39]

Conclusion

In this study, it was shown that MPO activity in kidney tissue of guinea pigs is affected from different magnetic field intensities applied in different periods. By the increase of EM field exposure, immune system is activated, therefore, increase of leukocyte migration and due to this fact increase in MPO activity can be observed.

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