ELF MAGNETIC FIELDS' EFFECTS ON LIPID PEROXIDATION IN LUNG AND KIDNEY

A.G. Canseven*, S. Coskun** and N. Seyhan*

* Department of Biophysics, Gazi University, Faculty of Medicine, Ankara, TURKEY
** Department of Biology, Gazi University, Faculty of Art and Science, Ankara, TURKEY

canseven@gazi.edu.tr

Abstract : The study was assessed to evaluate the influences of in vivo exposures to 50 Hz magnetic fields on lipid peroxidation of lung and kidney tissues in guinea pigs. Lipid peroxidation was quantified by measuring the formation of MDA. Fifty seven, 10 – 12 weeks old, male guinea pigs were used in this experiment. The subjects were divided into one control group and six experimental groups which were exposed to 50 Hz magnetic fields of 10 Gauss (G), 20 G and 30 G with the exposure periods of 4 hours /day and 8 hours/day for 5 days. MDA levels of lung and renal tissues were determined according to the Cassini et al.'s spectrophotometric method. Mann Whitney-U test was applied for statistical analysis. Decreased MDA levels were found in lung tissues for 8 hours daily exposures of 10 G and 30 G, whereas increased MDA level was found for 4 hours daily exposure of 10 G. MDA levels of kidney tissues were found increased under the effects of 10 G and 20 G magnetic fields applied the period of 4 hours/day.

Introduction

In modern society, humans have commonly exposed to electromagnetic (EM) fields in their homes or workplace by means of consumption of more electricity. Extremely Low Frequency (ELF) fields produced by power transmission lines, transformers and household appliances have been reported to cause various biological effects. In many epidemiological and laboratory studies, it has been suggested that there is a link between ELF magnetic field exposure and the increased incidence of certain types of tumor, particularly in leukemia and brain cancer [1-11]. A variety of EMF effects on biological and biochemical responses such as protein synthesis [12-16], gene expression and signal transduction [17,18], DNA damage [19-21], immune system functions [22-26], apoptosis induction [27], enzyme regulation [28-30], free radical activity [31-37] have been described in the reports of many in vivo and/or in vitro experiments. Magnetic fields penetrate the cells and can alter cell membrane potential and the concentration of ions [37-40]. These alterations may affect free radical processes within the cell. ELF EMF has been thought to prolong the life of free radicals and can act as a promoter or co-promoter of cancer [41].

Although oxygen is required for many important aerobic cellular reactions, it may undergo electron transfer reactions, which generate highly reactive membrane-toxic intermediates, such as superoxide, hydrogen peroxide or the hydroxyl radical. These reactive species inflict significant oxidative damage to membrane lipids. Oxidative DNA damage has been implicated in aging, carcinogenesis and other degenerative diseases [41].

It has been known that magnetic fields cause an increase in free radical activity in living organisms [37]. Free radicals are very reactive and unstable molecular species that can initiate chain reactions to form new free radicals [42]. Free radical formation induces changes in enzymes activity, gene expression, alteration of membrane structure and DNA damage [43].

Lipid peroxidation is the most common index of oxidative stress and malondialdehyde (MDA) is the last product of lipid peroxidation. In this investigation, we measured MDA levels of lung and kidney tissues of guinea pigs exposed to 50 Hz magnetic fields of 10 Gauss (G), 20 G and 30 G with the exposure periods of 4 hours /day and 8 hours/day for 5 days.

Materials and Methods

The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University (Report no: 36-7838). In this study, a total of 57 male, 250-300 g weighted (10-12 weeks aged) guinea pigs were used. The animals were fed standard pellet food and kept in the laboratory at a room temperature of 23±0.2 °C, a day and night cycle of 12 hours and ambient geomagnetic field of 0.3 G.

Exposures to Magnetic Fields

Magnetic fields were generated using two pairs of circular Helmholtz coils. Coil pairs of Helmholtz configuration were used in the vertical manner. Forty eight guinea pigs were housed in the centre of the Helmholtz coils, 2 per plastic cage and were exposed to 50 Hz, 10 G, 20 G and 30 G fields with the exposure periods of 4 hours/day and 8 hours/day for 5 days.

ISSN: 1727-1983

Animals were housed pairly in 26 x 22 x 10 cm^3 plastic cages positioned at the center of the energized Helmholtz coil during experiments, to avoid any distortion of the generated magnetic fields. Nine subjects were handled in an identical manner with the exposed animals in the same laboratory. They were housed at the center of the Helmholtz coils without being exposed to any magnetic fields and used as control. To control possible variation in responses due to circadian rhythm, daily exposure periods of 4 hours and 8 hours were chosen between 8:00-12:00 a.m. and 8:00 a.m. - 4:00 p.m. respectively.

After exposure periods, animals were sacrificed by ether inhalation in a closed box, then lung and kidney tissues were dissected out immediately. They shocked by liquid nitrogen and stored in deepfreeze at -40°C until performing the analysis of MDA contents. All experiments were run blind; i.e. the experimenters performing MDA assay did not know the exposure conditions of the animals.

Determination of MDA Levels of Kidney and Lung Tissues

MDA levels of lung and renal tissues were determined according to the Cassini et al.'s spectrophotometric method [44]. Lipid peroxidation was quantified by measuring the formation of thiobarbituric acid reactive substances (TBARS). Tissues were homogenized in nine volumes of cold 10 % TCA solution and the homogenate was centrifuged for 15 min. at 3000xg at 4°C. The supernatants were transferred to glass test tubes containing 0.375 % (w/v) thiobarbituric acid and 0.02 % (w/v) butylated hydroxytoluene to prevent further peroxidation of lipids during subsequent steps. The samples were then heated for 15 min at 100^0C in a boiling water bath, cooled and centrifuged to remove precipitant. The absorbance of each sample was determined at 532 nm.

Statistics

Statistical analyses were carried out using SPSS software (SPSS Inc., Chicago, USA). The P value was considered significant at $P<0.05$.

Mann Whitney-U and Kruskal-Wallis tests were used in statistical analysis. Comparisons between exposed groups and controls were made by using Mann Whitney-U test while magnetic fields of 10 G, 20 G and 30 G were compared with Kruskal-Wallis test with respect to exposure periods of 4 hours and 8 hours.

Results

In this study, MDA levels were determined in lung and kidney tissues of guinea pigs exposed to 50 Hz magnetic fields of 10 G, 20 G and 30 G. All of the MDA values given in the Table 1 are mean±standart error of mean.

Table 1: MDA levels of lung and kidney tissues of guinea pigs exposed to magnetic fields and controls

Tissues	*Groups*	*MDA Levels (nmol/g tissue)*
Lung		
	Control (n=7)	82.06 ± 5.16
	10 G, 4 hours (n=8)	107.99 ± 6.86 *
	10 G, 8 hours (n=8)	59.95 ± 3.33 *
	20 G, 4 hours (n=6)	84.70 ± 8.11
	20 G, 8 hours (n=7)	68.98 ± 4.36
	30 G, 4 hours (n=8)	66.56 ± 5.96
	30 G, 8 hours (n=9)	64.48 ± 4.30 *
Kidney		
	Control (n=7)	77.35 ± 6.54
	10 G, 4 hours (n=8)	104.53 ± 8.32 *
	10 G, 8 hours(n=8)	75.36 ± 4.00
	20 G, 4 hours (n=6)	160.00 ± 20.66 *
	20 G, 8 hours (n=7)	68.75 ± 2.40
	30 G, 4 hours (n=8)	67.20 ± 7.03
	30 G, 8 hours (n=9)	78.79 ± 5.61

* $p < 0.05$ with respect to controls

MDA levels were found increased with respect to controls in lung and renal tissues of guinea pigs exposed to the magnetic fields of 10 G and 20 G for 4 hours. The increases in MDA levels were found statistically significant for 10 G in lung tissue ($p=0.020$) and for 10 G ($p=0.021$) and 20 G ($p=0.002$) in renal tissues by Mann-Whitney U test. For 8 hours of exposure periods of 10 G, 20 G and 30 G, MDA levels in lung and kidney tissues were found reduced being more effective in lung tissue for 10 G ($p=0.008$) and 30 G ($p=0.027$).

Field intensities were compared with respect to exposure periods by Kruskal-Wallis test. Significant differences were determined between field intensities of 10 G, 20 G and 30 G in lung ($p=0.017$) and kidney ($p=0.001$) tissues for exposure period of 4 hours.

Decreases in MDA levels were estimated to be independent from the magnetic field intensities for lung and kidney tissues during 8 hours of exposure periods for 5 days. Magnetic field intensities of 10 G and 20 G seem to have opposite effect on MDA level with respect to 30 G for 4 hour exposure period. Statistically significant differences were observed between 10 G, 20 G and 30 G for 4 hours of exposure periods. Related p values were calculated to be $p=0.017$ and $p=0.001$ for lung and kidney tissues respectively.

Discussion

Most of the authors suggested that MF could increase the concentration of the free radicals in cells

ISSN: 1727-1983

and affect the biological systems by prolonging the life of free radicals [30, 45, 46].

MF may cause oxidative damage by reactive oxygen species (ROS) formation. ROS are extremely reactive and interact with all the macromolecules including lipids, nucleic acids and proteins. Particularly susceptible to oxidative damage by free radicals are the polyunsaturated fatty acid acyl chains of phospholipids, which lead to lipid peroxidation. Lipid peroxidation products e.g. TBARS has been taken as a biomarker to oxidative stress in biological system.

Recently, it was reported that MFs affect radical behavior and radical-pair intermediated enzymes activities [47-49]

In the present study, we examined the effect of MFs on kidney and lung homogenates by measuring the level of TBARS. Decreased MDA levels were found in lung tissues for 10 G and 30 G exposures with the exposure period of 8 hours/day whereas increased MDA level was determined for the magnetic field of 10 G applied with 4 hours/day. MDA levels of kidney tissues were found increased under the effects of 10 G and 20 G MF with the application period of 4 hour/day.

There are various studies in which the effect of magnetic field on free radical metabolism has been investigated. [50, 51].Watanabe et al (1997) measured lipid peroxidation, in the liver, kidneys, heart, lungs and brain of mice exposed to static magnetic fields. They found that lipid peroxidation of the kidneys, heart, lungs and brain did not change compared to control. Kashalda et al reported that magnetic field altered lipid peroxidation and increased ascorbic acid defense systems and depressed glutathione peroxidase and catalase in seminal tissue of rats. Jin et al 's results showed that SOD and GSH Px in whole blood significantly increased and the content of MDA decreased at exposure static magnetic fields of 1500 - 1550 G on human subject. Kula et al reported that cytosolic MDA levels increased significantly in both the liver and the kidney of female and male rats exposed to the ELF magnetic field. In female and male rats, liver MDA levels were higher than kidney MDA levels.

Our results were found are consistent with Kula's findings which was stating that MDA has been altered under the effect of electromagnetic fields.

Further studies will throw a new light on the mechanisms of effect created by magnetic fields in biological systems.

Conclusion

These results indicate that ELF EMF may modulate the oxidative stress of free radicals by enhancing lipid peroxidation and reducing some free radical scavengers. These alterations probably occur because of the difference of exposure systems, alternative or static MF, the frequency, intensity and exposure period of MF, the time of recovery, investigation targets and assay methods.

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ISSN: 1727-1983