Total antioxidant capacity, total oxidant status and oxidative stress index in the men exposed to 1.5 T static magnetic field

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Abstract. The aim of this study was to investigate the effects of a high-strength magnetic field produced by a magnetic resonance imaging (MRI) apparatus on oxidative stress. The effects of a 1.5 T static magnetic field on the total antioxidant capacity (TAC), total oxidant status (TOS) and oxidative stress index (OSI) in male subjects were investigated.

In this study, 33 male volunteers were exposed to a 1.5 T static magnetic field for a short time and the TAC, TOS and OSI of each subject were determined. Magnetic field exposure was provided using a magnetic resonance apparatus; radiofrequency was not applied. Blood samples were taken from subjects and TAC, TOS and OSI values were measured using the methods of Erel.

TAC showed a significant increase in post-exposures compared to pre-exposures to the magnetic field ($p < 0.05$). OSI and TOS showed a significant decrease in post-exposures compared to pre-exposures to a 1.5 T magnetic field (for each of two, $p < 0.01$).

The 1.5 T static magnetic field used in the MRI apparatus did not yield a negative effect; on the contrary, it produced the positive effect of decreasing oxidative stress in men following short-term exposure.

Key words: Strong magnetic field — Total antioxidant capacity — Total oxidant status — Oxidative stress

Introduction

In modern society, humans are frequently exposed to magnetic fields (MFs), including extremely low-frequency, low-intensity and high-intensity MFs. Low-intensity MFs are generally produced by power lines and many kinds of electrical appliances. High-intensity MFs are produced by apparatuses such as magnetic resonance imaging (MRI) equipment.

Several experimental and epidemiological studies have found an association between low-intensity MF exposure and the increased incidence of various types of cancer, including childhood leukemia, lymphomas, brain tumor and breast cancers (Jajte et al. 2001, 2002; Lee et al. 2004; Regoli et al. 2005).

Although several studies on cells have shown that MFs influence a large variety of cellular functions, the mechanisms of interaction of the MFs with living cells remain unclear (Lagroye et al. 1998; Jajte et al. 2001; Piacentini et al. 2001; Rollwitz et al. 2004). One of the potential mechanisms by which static MFs may interact with living organisms is through electronic interactions, i.e., radical-pair mechanisms (Jajte et al. 2001, 2002; Lee et al. 2004; Rollwitz et al. 2004; Regoli et al. 2005). Many of the proposed hypotheses assume that the cell membrane is the most likely target for the primary impact of the field, and that this interaction might affect changes in the intracellular levels of Ca^{2+} and the signal transduction mechanisms at different levels (Piacentini et al. 2001). MFs may be effective in enhancing the

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action of chemical mutagens, and cause damage to DNA (Baum et al. 1995).

Several *in vivo* and *in vitro* experiments have documented the occurrence of genotoxic effects after electromagnetic field (EMF) exposure which were not based on thermal effects but rather associated with oxyradicals (Czcy et al. 2004; Lai et al. 2004). Reactive oxygen species (ROS) are known for their cellular reactivity and toxic effects, including lipid peroxidation of membrane-bound organelles, protein degradation, enzyme inactivation, damage to DNA and more complex states of disease originating from interactions and indirect effects (Wolf et al. 2005). ROS can act as messengers in many cellular cascades and regulatory processes, including intracellular homeostasis of calcium, with significant implications for signal transduction pathways, cell proliferation, and oncogene expression (Löscher and Liburdy 1998).

The radical-pair mechanism was suggested by Brocklehurst and McLaughlan (1996) as a possible route, whereby a MF of environmental strength might affect a biological system. Lalo et al. (1994) and Kubato (2001) suggested that a steady MF could accelerate lipid peroxidation. Fiorani et al. (1997) reported that a MF (50 Hz, 0.5 mT) increased damage in an oxidative-stressed rabbit erythrocyte system.

Static MFs may interact with biological systems. One of the potential mechanisms by which SMF may interact with the living organism is through electronic interactions, i.e. radical pairs mechanism. MFs influence the kinetics of reactions with radical pair intermediates. External MFs can increase the concentration of free radicals in living cells. MF 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. When ROS recat with nonradicals, new free radicals can be formed resulting in chain reactions, i.e. lipid peroxidation (Grissom et al. 1995).

Safety issues and discussions about potential hazards associated with MRI systems and procedures have been extremely controversial over the past decade: partly because of the disputed assertions about the role of EMFs in carcinogenesis or the promotion of abnormalities in growth and development, partly because the assumption that MRI was an inherently safe procedure had reduced the importance of the publication of negative results. Since the introduction of MRI as a clinical modality in the early 1980s, more than 100,000,000 diagnostic procedures (estimated) have been completed worldwide, with relatively few major incidents (Prato et al. 1997).

A comprehensive presentation and discussion of MRI-related hazardous effects is beyond the scope of this paper; thus, we will limit the present discussion to bio-effects produced by MRI systems acting directly on the human body. Several research studies have been conducted over the past thirty years in order to assess the potential dangerous bio-effects associated with exposure to MRI diagnostics. Because of the complexity and importance of this issue, most of these works are dedicated to separately examining biological effects produced by a particular MF or EMF source utilized in MRI. Moreover, the scientific literature contains an ever-increasing number of studies concerning the biological effects produced by the interactions of biological matter with EMFs. Thus, there is a need to integrate and summarize the current findings germane to this topic and, at the same time, to provide basic data that will contribute to our understanding of the physics of the interactions between EMFs and biological systems (Formica et al. 2004).

In the range of Tesla, the effects of high-intensity MFs on total antioxidant and oxidative stress have not been investigated. Thus, in the present study, we have investigated the effects of a 50 Hz, 1.5 T high-intensity static MF on total antioxidant capacity (TAC) and oxidative stress index (OSI).

Materials and Methods

Application of a MF

The research was designed and implemented according to the principles of the Declaration of Helsinki. This experimental research was performed with an approval of ethics committee of Medicine Faculty of Harran University. Thirty-three male volunteers were subjected to a 1.5 T MF for 30 min, and the TAC and total oxidant status (TOS) of each subject were measured, so that their respective OSI could be determined. The MF exposure was performed using a MRI device (Picker Edge, 1.5 T, AFE 126, American Production, 2001 upgrade) in which the volunteers were kept for a period of 30 min each. A radiofrequency was not applied, only the 1.5 T MF. The room temperature of 22°C and a relative humidity of 45% were maintained throughout the application. The MF was applied to each subject at the same time of day. 5-ml samples of blood were acquired from the volunteers into citrated tubes, both 1 min before the application of the MF and 1 min after. The blood samples were centrifuged for 8 min at a rate of 5000 revolutions/min, and plasma was separated.

Measurement method for oxidative stress

TAC measuring method

The plasma was analysed for TAC, thiol, ascorbic acid, uric acid, bilirubin, total protein and albumin. The TAC levels were determined by two different and novel automated methods developed by Erel (2004). In the first method (TAC 1), the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colourless substrate *o*-dianisidine to produce the dianisyl

radical, which is bright yellowish-brown. The assay results are expressed in millimole Trolox equivalent units *per* litre, and the precision of this assay is excellent – less than 3%. Total peroxide concentrations of the plasma were determined by the FOX2 method with minor modifications. The FOX2 test system is based on the oxidation of ferrous iron to ferric iron by various types of peroxides contained in the plasma samples, in the presence of xylenol orange, which produces an orange-coloured ferric-xylenol complex whose absorbance can be measured. Aliquots (200 μl) of plasma were mixed with 1.8 ml of the FOX2 reagent.

After incubation at room temperature for 30 min, the vials were centrifuged at 12,000 × *g* for 10 min. The absorbance of the supernatant was then determined at 560 nm. The total peroxide contents of the plasma samples were determined as a function of the difference in absorbance between the test and blank samples, with a solution of H_2O_2 used as a standard. The coefficient of variation for individual plasma samples was less than 5%. Plasma total protein, albumin, uric acid and bilirubin levels were measured using commercial kits (Abbott Laboratories). Vitamin C concentration was measured by the FRASC method. The percent ratio of the total peroxide to the TAC yields OSI 1, an indicator of the degree of oxidative stress.

TOS measuring methods

Oxidant present in the sample oxidizes the ferrous ion-*o*-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion produces a coloured complex with xylenol orange in an acidic medium. The colour intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar H_2O_2 equivalent *per* liter, and the reading was taken at 450 nm.

Subsequently, the plate was incubated for 20 min in the dark. After this, the reaction was stopped with 50 μl of 2N H_2SO_4, and the second absorbance reading was taken at 450 nm. The total peroxide levels of the samples were calculated as the differences between the absorbance readings related to the H_2O_2 standard curve (Erel 2005).

OSI

The percent ratio TOS to TAC was accepted as the OSI, an indicator of the degree of oxidative stress. To perform the calculation, we changed the resulting unit of TAC, millimoles of Trolox *per* litre, to micromoles *per* litre, and the OSI value was calculated from the formula: OSI = [TOS μmol/l / TAC μmol of Trolox X 100].

Statistical analysis

Data were analyzed by paired-sample Student's *t*-tests. Pre- and post-exposure values were compared to one another. *p* values below 0.05 were considered to be statistically significant.

Results

TAC showed a profound increase in post-exposures compared to pre-exposures to the MF ($p < 0.05$). TOS and OSI show a profound decrease in post-exposures compared to pre-exposures to the 1.5 T MF (for each of two, $p < 0.01$). The results are given in Table 1.

Discussion

Extremely low-frequency MFs (ELF-MFs) have been the subject of public debate for some time, but whether exposure at power intensities of 50 and 60 Hz represents a hazard to human health is still open to question. At present, no epidemiological evidence has supported the widely accepted association with cancer process, and the assessment of the biological impact of ELF-MFs remains a complex issue (Mc-

Table 1. Total antioxidant capacity (TAC), Total oxidant status (TOS) and Oxidative stress index (OSI) in pre-exposure and post-exposure of 1.5 T magnetic field. TAC was analysed from plasma, antioxidants as thiol, ascorbic acid, uric acid, bilirubin, total protein and albumin. Oxidant present in the sample oxidizes the ferrous ion-*o*-dianisidine complex to ferric ion. OSI is accepted as the percent ratio TOS to TAC. OSI = [TOS μmol/l / TAC μmol of Trolox X 100]

		TAC* (μmol/l)			TOS** (μmol/l)			OSI***		
	n	mean	SD	SEM	mean	SD	SEM	mean	SD	SEM
Pre-exposure	33	1.19	0.17	0.32	22.53	9.49	1.79	1.99	0.97	0.18
Post-exposure	33	1.27	0.16	0.30	18.67	9.11	1.72	1.55	0.89	0.16

SD, standard deviation; SEM, standard error of the mean; * $p < 0.05$; ** and *** $p < 0.01$.

Cann et al. 1998; Crupton et al. 2004). In this regard, several investigations have been directed toward understanding the cellular-molecular mechanisms by which MFs interact with biological systems, possibly facilitating the initiation of carcinogenesis and the onset of long-term effects (Lacy-Hulbert et al. 1998).

Enhanced pro-oxidant conditions and free radical formation have been suggested in different biological models as an important pathways of response induced by EMFs, which modulate both the turnover of oxyradicals and cellular effects, including cell proliferation, induction of ROS-generating enzymes, signal transduction processes, modulation of proto-oncogenes, and genotoxicity (Fernie et al. 2001; Rollwitz et al. 2004). Following lipid peroxidation processes and calcium leakage from internal storage then trigger the activity of nitric oxide synthase and the release of NO, responsible for damages to DNA and other macromolecular districts (Lai and Singh 2004).

Various mechanisms have been postulated for oxidative damage, but different pathways can overlap with both indirect and cascade effects, and the same pro-oxidant stressor can interact with several targets and with different actions (Regoli et al. 2002; Gorbi and Regoli 2003). Those studies suggested that ELF-MFs adversely affect biological systems at the molecular level. However, the MFs used in those studies were at the levels of μT and mT and were long-term.

With regard to MFs, pulsed MFs in particular have been used for magnetotherapeutic aims, e.g., bone capture therapy, wound healing and nerve regeneration (Model et al. 1997; Yan et al. 1998; Kinney 2005). In our previous study, we observed that low-intensity ELF-MFs (50 Hz, 1 mT) decreased the fatty-acid composition of the phospholipid fraction of rat testes (Sert et al. 2002). Yokus et al. using analysis of steady-state levels of 8OHdG (8-hydroxydeoxyguanosine) determined that ELF-MFs caused oxidative DNA damage (Yokus et al. 2005).

Our study has yielded striking results. Oxidative stress decreased in men exposed to a 1.5 T static MF for 30 min, resulting in increases in TAC and decreases in TOS. These results are in contrary to results from extremely low-frequency and low-intensity (μT and mT) MFs. However, our results have shown that a 1.5 T static MF does not have adverse effects on biological systems.

It was suggested that MFs have been shown to stabilize free radical species, thus increasing their overall concentration and dispersion within the cell and, hence, the probability of oxidative injury (Scaiano et al. 1994). Further, it has been reported that low-intensity MFs (μT and mT) increased the cellular lifespans of ROS. Oxyradical-mediated effects of EMFs have been reported in rats exposed to 0.01 mT (Lai and Singh 2004). MFs can promote a Fenton-like reaction with the formation of hydroxyl radicals, which damage lipids, proteins, DNA and, in turn, calcium homeostasis (Lai and Singh 2004).

Rollwitz et al. (2004) have shown significant increases in ROS production (20%) and in superoxide anion radical release (25%) in MF-exposed (50Hz, 1 mT) promonocytes. Regoli et al. (2002) observed that the activities of catalase and glutathione reductase and superoxydedismutase enzymes decreased when exposed to 50 μT MF, and TAC and ROS level increased with exposure to the same intensity of MF (Regoli et al. 2005). Grissom demonstrated that a 5 mT static MF increased lipid peroxidation in isolated rat liver microsomes (Grissom 2005).

The results of our study are interesting insofar as they conflict with the results of other studies. However, the MFs used in other studies were low-intensity ELF-MFs. The MF used in our study was 1.5 T and of high intensity. A 1.5 T MF increased the TAC in men.

We suppose that high-intensity MFs may shorten the lifespans of ROS and other free radicals. In addition, ROS may have some signalling adaptive properties resulting in downregulation of oxidative stress (Squier 2001; Bigelow and Squier 2005). More probably, signal function of ROS is responsible for increased antioxidant capacity and decrease in TOS.

This result shows that a 1.5 T static MF generated by an MRI apparatus does not produce negative effects in men; on the contrary, it decreases oxidative stress.

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Final version accepted: January 18, 2007