Effects of Static and 50 Hz Alternating Electric Fields on Superoxide Dismutase Activity and TBARS Levels in Guinea Pigs

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Abstract. The toxic oxygen free radicals are extremely reactive and can cause considerable damage to biomolecules, such as RNA, enzymes, membranes, proteins, and lipids, which may in turn lead to various pathological consequences. Lipid peroxidation, evaluated by determination of thiobarbituric acid reactive substances (TBARS) is the free radical-induced oxidation of polyunsaturated fatty acids. Normally, the oxygen free radicals are neutralized by highly efficient systems in the body. These include antioxidant enzymes like superoxide dismutase (SOD). In a healthy subject, there is a balance between free radicals and levels of antioxidants. The aim of this study was to determine lipid peroxidation and SOD levels in plasma, liver, lung and kidney tissues exposed to different intensities, directions and exposure periods of static and 50 Hz alternating electric fields. Electric field intensities ranging from 0.3 kV/m to 1.8 kV/m were applied in vertical or horizontal direction in exposure periods of 1, 3, 5, 7, and 10 days. The increase in SOD and TBARS levels of plasma, liver, lung, and kidney tissues was found to depend significantly on the type of electric field and the exposure period.

Key words: Electric field — Radical synthesis — Lipid peroxidation — Antioxidant — Superoxide dismutase — Thiobarbituric acid reactive substances

Introduction

Electric fields affect selective transport of ions or molecules through membranes (Benov et al. 1994; Blumenthal et al. 1997; Barriviera et al. 2001; Ivancsits et al. 2003). One approach to investigations of effects of electric and magnetic fields on biological systems is to compare the impact of man-made sources to those to which

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we are naturally exposed (van Wijngaarden et al. 2000; Kaune et al. 2002; Qiu et al. 2004). Natural electric fields at the surface of the Earth have both static and alternating components. Static electric fields occur because of the polarity between the surface of the Earth and the upper atmosphere and are about 130 V/m near the Earth's surface. Alternating electric fields decrease rapidly with increasing frequency in the range of 0–300 Hz and originate from thunderstorms and geomagnetic pulsations that produce currents within the Earth. Short pulses of magnetohydrodynamic origin generate 0.2 to 1000 V/m electric fields in the frequency range of 0.001 to 5 Hz (Grandolfo and Vecchia 1985; Repacholi and Greenebaum 1999). We spend a large part of our lives surrounded by a grid of wires that deliver energy we need to power lights, electric motors, and the numerous appliances that facilitate modern life; majority of our exposure comes from fringing fields generated by the power distribution systems.

Participation of free radical biochemistry in several pathologies and aging has been demonstrated. Free radicals, such as superoxide anions ($O_2^{\bullet-}$), generated by electrical stimuli show high chemical reactivity and, as a result, have a relatively short lifetime in the free state (Stevens 2004; Harakawa et al. 2005; Yokus et al. 2005; Bediz 2006). Free radical oxidation of polyunsaturated fatty acids in biological systems is known as lipid peroxidation, and detection and measurement of lipid peroxidation is the evidence most frequently cited to support the involvement of free-radical reactions (Hallivell et al. 2000). Malondialdehyde (MDA) is a breakdown product of major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress. The thiobarbituric acid (TBA) method was employed to analyze plasma MDA levels. Free radicals are very reactive and unstable molecular species that can initiate chain reactions to form new free radicals. Although formed as a result of a wide range of normal biochemical processes, they are potentially damaging. Several mechanisms are in place to neutralize their effects, which include a system of nutritional and endogenous enzymatic antioxidant defenses that generally hold the production of free radicals and prevent oxidant stress and subsequently tissue damage (Chan 2001; Moustafa et al. 2001).

The importance of the enzyme superoxide dismutase (SOD) in eliminating these radicals is well established (Desideri et al. 1992). SOD scavenges the superoxide radicals by catalyzing the reactive $O_2^{\bullet-}$ species into dioxygen and oxygen peroxide, thereby protecting cells against the reactive oxygen species (ROS) produced by electric fields or other mechanisms (Benov et al. 1994; Scaiano et al. 1994; Irmak et al. 2002; Lee et al. 2004; Güler et al. 2005; Harakawa et al. 2005; Ozguner et al. 2005).

This study investigated the changes in radical synthesis due to the effects of static and 50 Hz extremely low frequency (ELF) electric fields, similar to those to which we are exposed in daily life. Changes as a function of magnitude, direction and exposure period were studied.

Materials and Methods

Exposure system

Guinea pigs were housed in wooden cages with dimensions of $50 \times 50 \times 14$ cm and were exposed to both static and ELF electric fields. For vertical field exposure, copper plates were mounted on the top and bottom faces of the cages to form parallel plates of a capacitor. The copper plate spacing was 14 cm and the dimensions of the plates were $50 \times 50 \times 0.1$ cm. The positive terminal of the power supply was always connected to the upper plate and the negative terminal to the lower plate. For horizontal field exposure, the copper plates were mounted on the left and right faces of the cages. The positive terminal of the power supply was always connected to the left plate and the negative terminal to the right plate. Potential differences were controlled continuously throughout the experiment and were kept constant with the aid of a 3-digit LED display of power supply voltage (Teta T-994 DC&AC). Also, a multi-meter connected to the circuit was used to double-check the potential difference between the parallel plates. The arrangement was chosen so as to keep the distance between the copper plates small with respect to their dimensions in order to generate homogeneous electric field in the exposure space. The magnitude of the electric field in the cages of guinea pigs was determined not only by theoretical calculation, but also by measurements using a Narda EFA-300 electric field probe.

Exposure to static electric field

Electric potentials were applied to the copper plates mounted on the wooden boxes to produce electric fields with magnitudes of (in kV/m): 0.3, 0.6, 0.8, 1, 1.35, 1.5, and 1.8. Male white guinea pigs (150–200 g) were continuously exposed to both horizontal and vertical electric fields for 8 h a day during 3 days. A total of 140 guinea pigs were exposed to electric fields. The animals were divided into 14 separate groups of 10 and each group was subjected to a specific combination of electric field intensity and direction. Each group was exposed to the electric field from 9 a.m. to 5 p.m. Twenty guinea pigs were used as controls and were kept under the same conditions without being exposed to any electric field. Animals were housed in cages for 3 days.

Exposure to ELF electric field

Five groups of 15 male white guinea pigs (150–200 g) were exposed to 50 Hz, 1.35 kV/m vertical electric fields. Each group was exposed daily for 8 h for 1, 3, 5, 7, or 10 days. The electric field exposure time was from 9 a.m. to 5 p.m. 75 guinea pigs were exposed according to the exposure period while 20 guinea pigs, which were not exposed to any electric field, formed a control group.

Other experimental features

Approximately 8 week old male guinea pigs, weighing 150–200 g, were obtained from the Turkish Hıfzıssıha Institute. All guinea pigs, exposed and controls, were examined and kept under surveillance of a veterinary in the Gazi University Laboratory Animals Breeding and Experimental Research Center. Since placing more than one animal in a cage would create a stress factor, only one animal was placed in each cage during each electric field exposure period. All the animals were kept at room temperature of 23 ℃, relative humidity of 50 %, were on a day and night cycle of 12 h and were fed *ad libitum* on a standard lab chow and carrot.

Biochemical evaluation

After the last exposure day, the guinea pigs were killed by decapitation. Immediately after decapitation, all tissues were removed and eluting blood collected for further analyses. Heparin was used as anticoagulant. Plasma was kept at −80 ℃ until analysis. Tissues were washed with cold saline solution and stored at −30 ℃ until processing. Tissues were homogenized in four volumes of ice-cold Tris-HCl buffer (50 mmol/l, pH 7.4) using a homogenizer (Disperser T10 basic D-79219, IKA-WERKE, GmbH, Staufer) after cutting the tissues into small pieces with scissors (for 2 min at 5000 rpm). Thiobarbituric acid reactive substances (TBARS) levels were determined at this stage. The homogenate was then centrifuged (Mikro 22/22R, Hettich-Zentrifugen GmbH&Co.) at $5000 \times g$ for 60 min to remove debris. The supernatant solution was extracted with an equal volume of an ethanol/chloroform mixture (5/3 volume *per* volume). After centrifugation at $5000 \times g$ for 30 min, the clear upper layer (the ethanol phase) was taken and used in determination of SOD activity and protein assays.

Tissue TBARS assay

The extent of lipid peroxidation was evaluated on tissues by spectrophotometrical measuring of the concentration of TBARS (based on the method of Beuge). Tissue samples were weighed and homogenized in cold 1.15 % KCl to make 10 % homogenate. 0.5 ml of the 10 % homogenate was pipetted into a 10 ml centrifuge tube to which 3 ml of 1 % phosphoric acid and 1 ml 0.6 % TBA aqueous solution were added. The mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml n-butanol was added and mixed vigorously. The butanol phase was separated by centrifugation and absorbance was measured at 535 and 520 nm (UV-1601 Shimadzu spectrophotometer, Japan). The difference was used as the TBARS value. Results were expressed in nanomoles *per* milligram protein (Beuge and Aust 1978).

Plasma TBARS assay

Plasma levels of lipid peroxidation products were assessed in freshly drawn samples by means of the TBA reaction, according to Yoshioka et al. (1979). Results were expressed in micromoles *per* litre. Concentration of TBARS, comprising mostly

lipid peroxidation products, was determined from absorbance at 532 nm, with tetramethoxypropane as a standard.

Tissue SOD analysis

Total SOD activities were measured by the nitroblue tetrazolium (NBT) inhibition assay (Sun et al. 1988). This assay is based on measurement of total SOD activity. The protein concentration was measured by the method of Lowry et al. (1951). Results were expressed in units *per* milligram protein.

Plasma SOD analysis

Total plasma SOD activities were measured using a modification of Sun's method. The SOD activity unit was defined as the amount of enzyme protein that resulted in 50 % inhibition in NBT. The reduction rate and results were expressed in units *per* milliliter enzyme solution for SOD (Sun et al. 1988).

Statistical analysis

Student's *t*-test was used to compare the MDA and SOD contents of tissues for each group exposed to the electric field and their controls. The results and significance levels are given in Figs. 1–6.

Results

Static electric field

Effects of static electric fields of different strengths and directions on TBARS levels and SOD activities in plasma, liver, lung, and kidney tissues were investigated (Figs. 1–4). It was found that TBARS levels and SOD activities in plasma were increased under both vertical and horizontal electric fields as compared to control group. Although the increases due to exposure to 0.3 and 0.6 kV/m electric fields were statistically insignificant ($p > 0.05$), the increase in TBARS levels and SOD activities for the 0.8, 1, 1.35, 1.5, and 1.8 kV/m electric fields were statistically significant ($p < 0.001$). In liver and kidney tissues, the increases in TBARS levels and SOD activities for the 1, 1.35, 1.5, and 1.8 kV/m electric fields were statistically significant ($p < 0.001$). The threshold magnitude of electric field that produces a statistically significant ($p < 0.001$) increase in lung tissue was 1.35 kV/m for TBARS and a statistically significant increase in SOD activity was found in lung tissues of animals exposed to 1 kV/m or stronger electric fields (with respect to controls).

ELF electric field

The effect of 50 Hz, 1.35 kV/m electric field on TBARS and SOD levels was investigated for different application periods (Figs. 5–6). Plasma levels of TBARS and SOD activities in animals exposed to 50 Hz, 1.35 kV/m electric field for one or several days were significantly increased with respect to controls ($p < 0.05$). It was
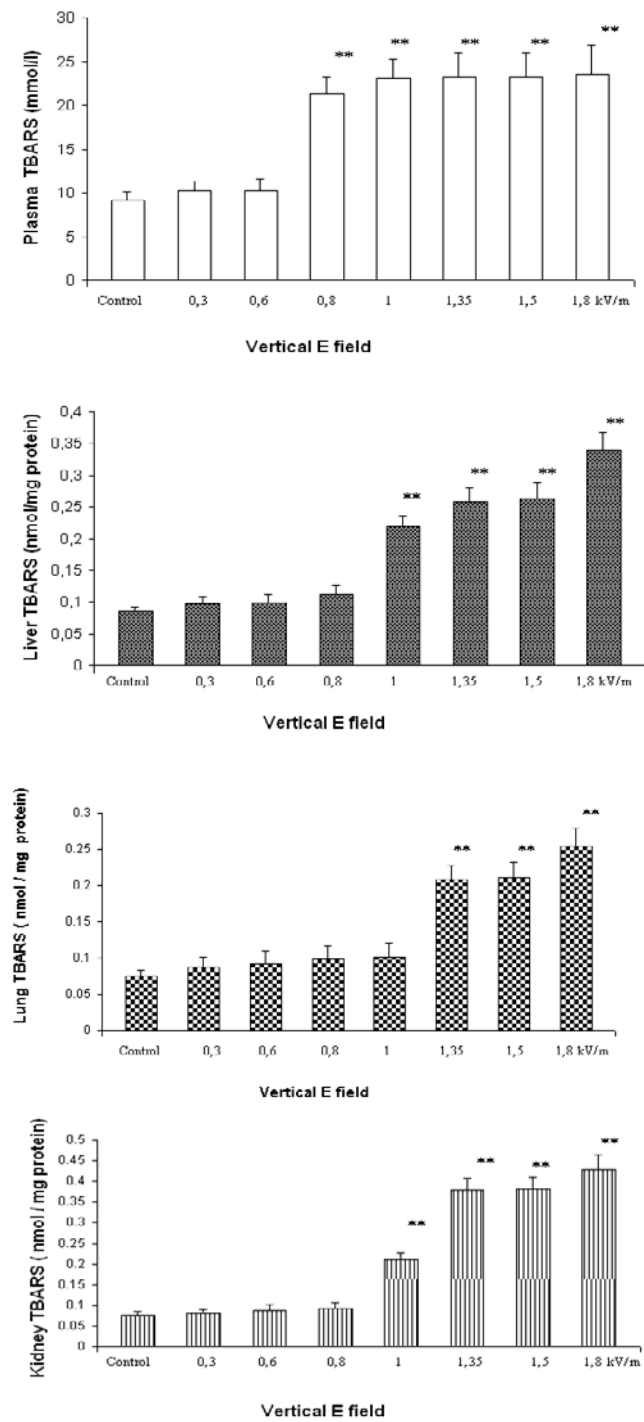
Figure 1. TBARS level of plasma (μmol/l), liver, lung and kidney tissues (nmol/mg protein) of vertical electric (E) field exposed and control groups. Guinea pigs were exposed to E fields between 0.3–1.8 kV/m for 3 days, 8 h/day (between 9 a.m. and 5 p.m.) in wooden cages. ** $p <$ 0.001.
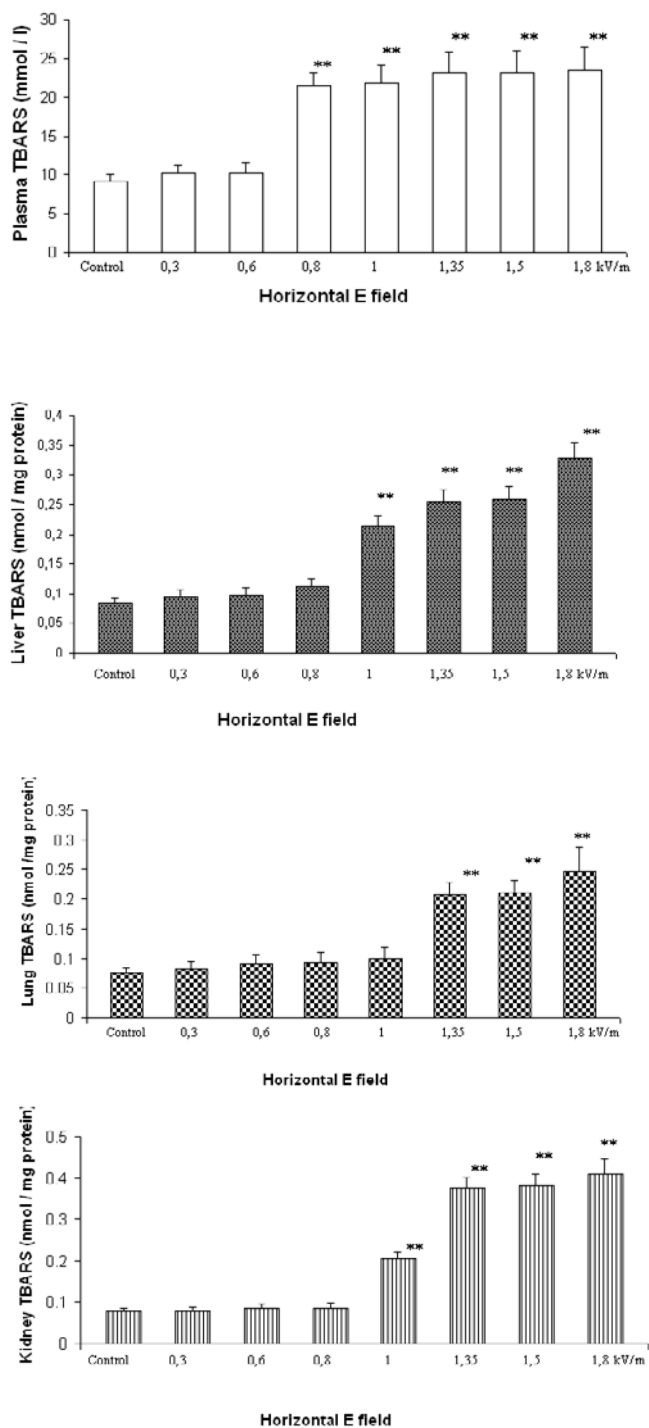
Figure 2. TBARS level of plasma (μmol/l), liver, lung and kidney tissues (nmol/mg protein) of horizontal electric (E) field exposed and control groups. Guinea pigs were exposed to E fields between 0.3–1.8 kV/m for 3 days, 8 h/day (between 9 a.m. and 5 p.m.) in wooden cages. ** $p < 0.001$.
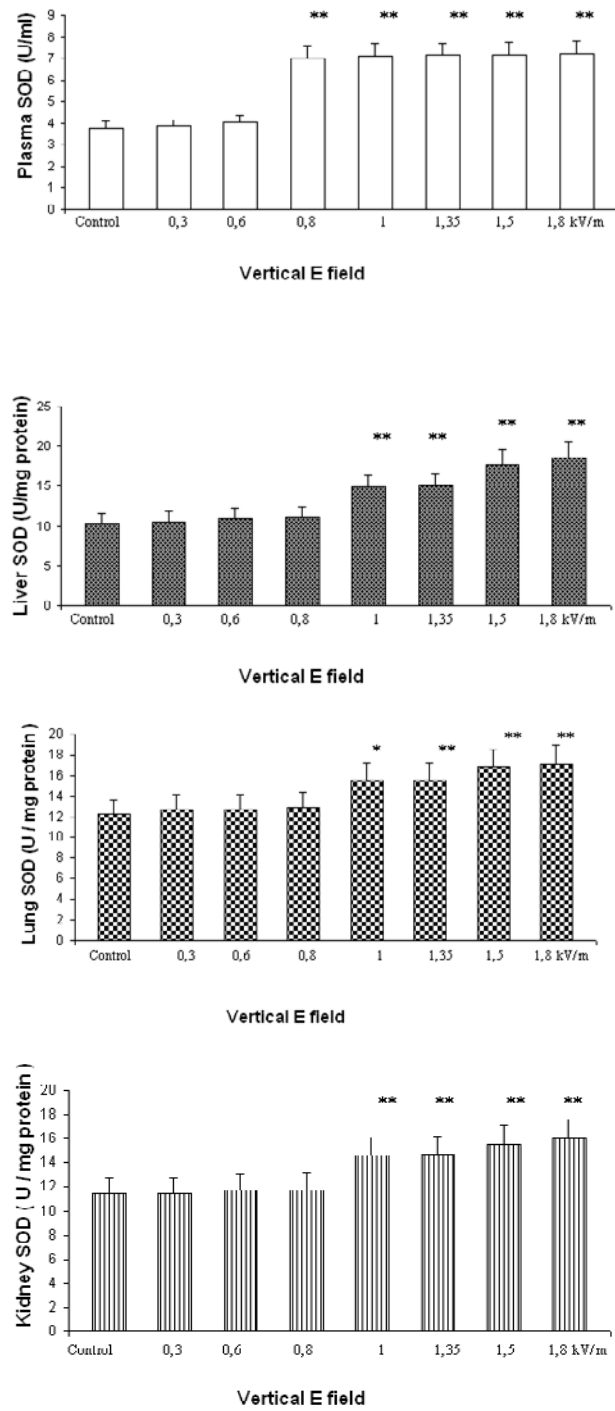
Figure 3. SOD level of plasma (U/ml), liver, lung and kidney tissues (U/mg protein) of vertical electric (E) field exposed and control groups. Guinea pigs were exposed to E fields between 0.3–1.8 kV/m for 3 days, 8 h/day (between 9 a.m. and 5 p.m.) in wooden cages. * $p < 0.05$, ** $p < 0.001$.

Figure 4. SOD level of plasma (U/ml), liver, lung and kidney tissues (U/mg protein) of horizontal electric (E) field exposed and control groups. Guinea pigs were exposed to E fields between 0.3–1.8 kV/m for 3 days, 8 h/day (between 9 a.m. and 5 p.m.) in wooden cages. * $p < 0.05$, ** $p < 0.001$.
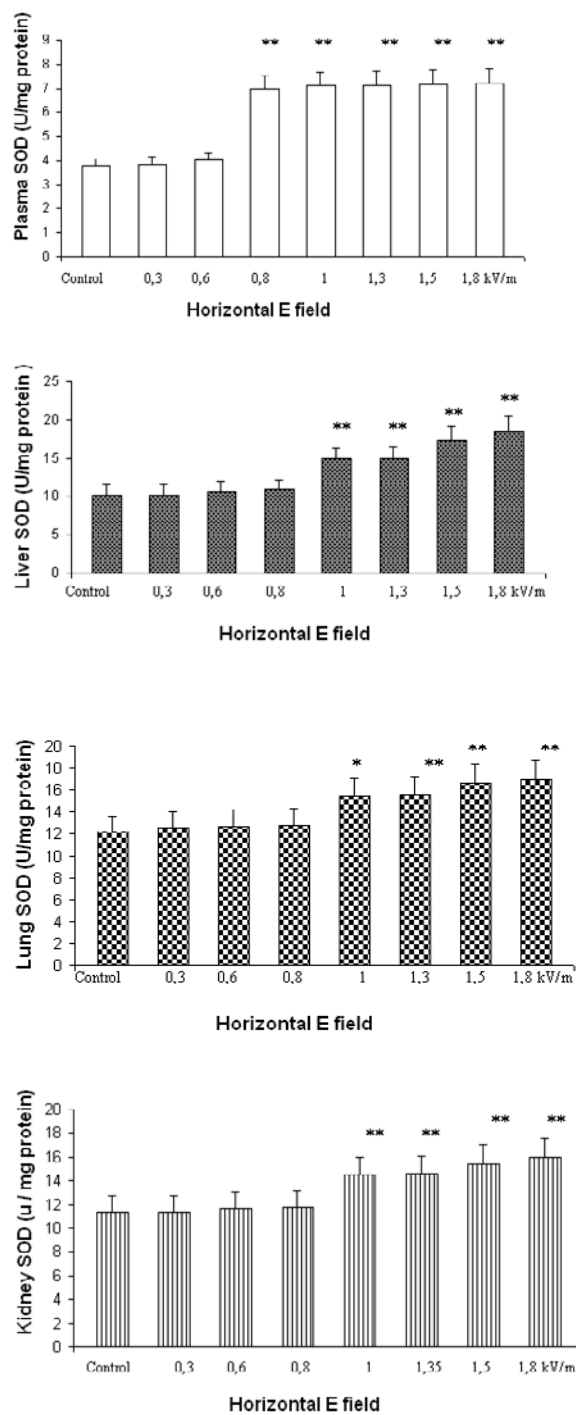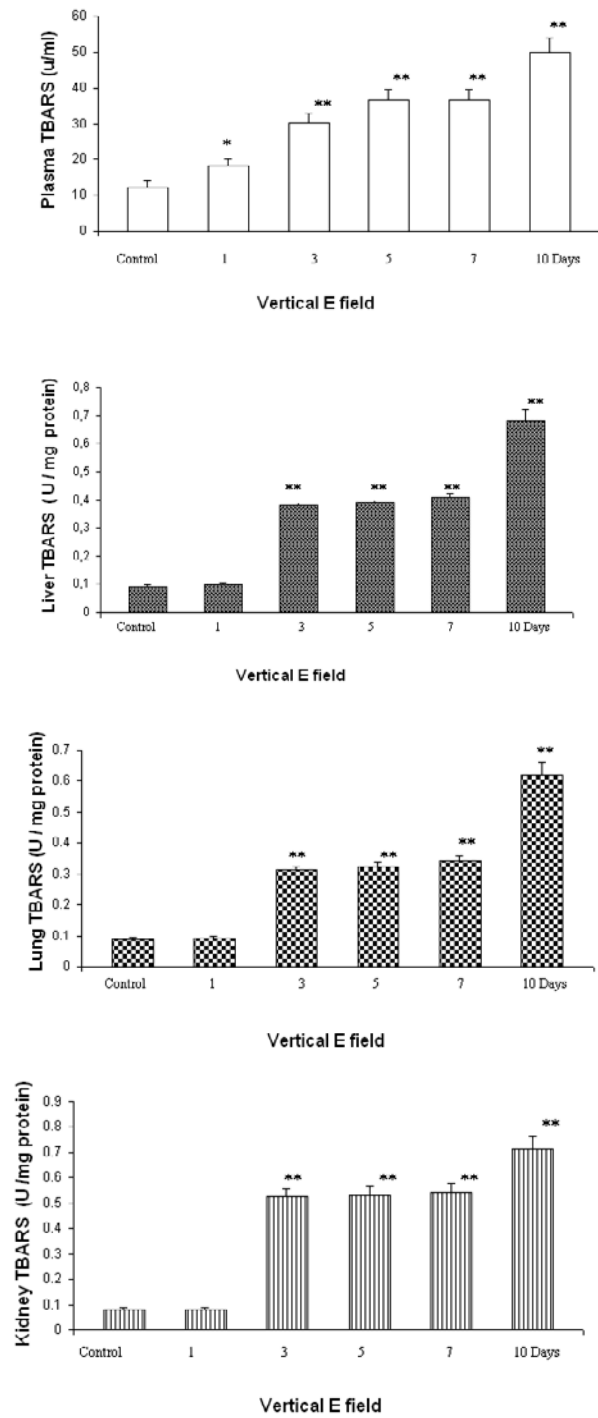
Figure 5. TBARS level of plasma (μmol/l), liver, lung and kidney tissues (nmol/ mg protein) of 50 Hz, 1.35 kV/m vertical electric (E) field exposed and control groups. Exposure period was 8 h/day for 1, 3, 5, 7 and 10 days through the study. * $p < 0.05$, ** $p < 0.001$.

Figure 6. SOD level of plasma (U/ml), liver, lung and kidney tissues (U/mg protein), of 50 Hz, 1.35 kV/m vertical electric (E) field exposed and control groups. Exposure period was 8 h/day for 1, 3, 5, 7, and 10 days through the study. * $p < 0.05$, ** $p < 0.001$.
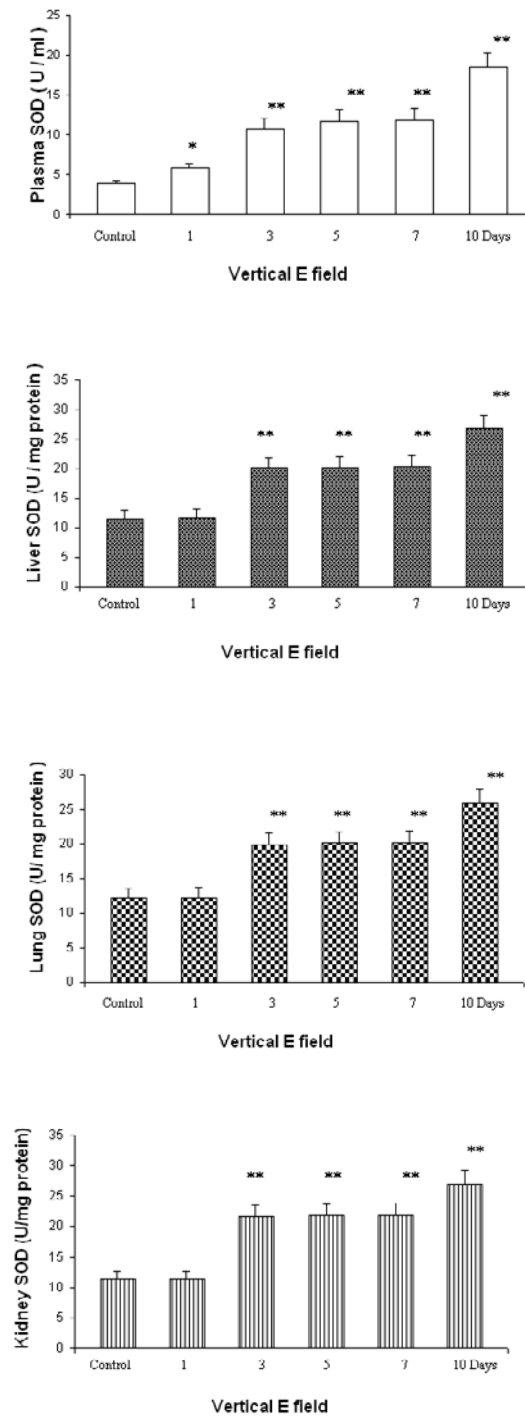
found that 3, 5, 7 and 10 days of exposure made both parameters increase even further ($p < 0.001$).

Pairwise comparisons of plasma TBARS levels in exposure groups show significant differences, with the exception of the 5 and 7 days exposure groups. Pairwise differences in plasma SOD activities between the 3 and 5 days, 3 and 7 days, and 5 and 7 days exposure groups were not statistically significant ($p > 0.05$), but the differences between the other exposure groups were significant.

TBARS levels and SOD activities in liver, lung, and kidney tissues were significantly increased in groups with 3 days and longer exposure, with respect to controls ($p < 0.001$). Pairwise comparisons of exposure groups in all tissues revealed significant differences in TBARS levels and SOD activities between all group pairs except the 3 and 5 days, 3 and 7 days, 5 and 7 days groups.

Discussion

Exposure to low-frequency electromagnetic field (EMF) (0–300 Hz) can lead to cell death as a result of increase in free oxygen radicals and DNA damage (Cossarizza et al. 1993; Benov et al. 1994; Ivancsits et al. 2003; Bediz et al. 2006). Despite available literature on the effects of static and 50 Hz electric fields on biological systems, our understanding of the underlying mechanisms at the molecular level is extremely poor. One hypothesis, based on well-established observations, is that both static and time-varying electric and magnetic fields can increase the yields of some types of free oxygen radicals such as $O_2^{\cdot-}$ and OH^- (Desideri et al. 1992; Ivancsits et al. 2003; Lee et al. 2004; Yokus et al. 2005).

Oxidative stress occurs when intracellular antioxidant mechanisms are overwhelmed by ROS (Hallivell and Gutteridge 2000). The role of ROS in tissue injury has been assumed. It has been demonstrated in numerous studies that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues (Ozguner et al. 2006). The main ROS that have to be considered are $O_2^{\cdot-}$, which are predominantly generated by the mitochondria, hydrogen peroxide (H_2O_2) produced from $O_2^{\cdot-}$ by the action of SOD, and peroxynitrite ($ONOO^-$), formed when $O_2^{\cdot-}$ couples with nitric oxide (NO) (Irmak et al. 2002). ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (CAT).

SODs are specific antioxidant enzymes that dismutate $O_2^{\cdot-}$, forming H_2O_2. Three SODs, the copper/zinc SOD (cytosolic SOD), manganese SOD (mitochondrial SOD), and extracellular SOD are the major antioxidant enzymes based on cellular distribution and localization. Of the three isoforms, extracellular SOD may be the most important in blood vessels, accounting for up to 70 % of the total SOD activity in blood vessels (Oury et al. 1996; Irmak et al. 2002). Total (Cu/Zn and Mn) SOD activity was determined according to the method of Sun et al. (1988) with a slight modification by Durak et al. (1993). Briefly, the method is based on inhibition in NBT reduction by the xanthine/xanthine oxidase system as a super-

oxide generator. In our study, total SOD activities were detected in plasma and selected tissues.

In this investigation, statistically significant differences were found between the TBARS and SOD contents of plasma and tissues of liver, lung and kidney of the groups exposed to static and 50 Hz electric fields and those of the control groups (Figs. 1–6). Increase in the intensity of the static electric fields resulted in an increase in the TBARS and SOD levels detected in all tissues (Figs. 1–4). For static electric field exposure, statistically significant elevations in TBARS and SOD levels were found starting at 0.8 kV/m for plasma, and at 1 kV/m for liver, lung, and kidney tissues. As such in our findings, static fields of 1 kV/m have been shown to move protein molecules along membrane surface and through gap junctions (Cevc et al. 1981). Also other experimental findings, such as lining of rod cells in retina up the direction of applied 0.8 kV/m electric field, changes in enzymatic activity of N-acetyl transferase and concentration of pineal melatonin under the effect of electric fields in the range of 1.2 to 1.9 kV/m, effects of electric fields between 0.6 and 2 kV/m on albumin level increase, combined with decrease in α, β, γ globulin, changes in circadian locomotors' activities of *musca domestica* flies under the effect of 1 kV/m electric field, or increase in mortality of mice exposed to 1.2 kV/m electric field indicate that electric fields in the range of 1 kV/m may be threshold level for producing adverse biological and health effects in living organisms (Moos et al. 1964; Marino 1983; Wilson et al. 1986; Kovacs et al. 1992; Lee et al. 1993; Engelmann et al. 1996). In another study, we presented histological and biochemical findings indicating that electric fields in the range of 0.9–1.9 kV/m affect collagen synthesis (Güler and Seyhan Atalay 1996; Güler et al. 1996). Benov showed that TBARS levels increased proportionally with the intensity of electric field (Benov et al. 1994).

An important parameter that has to be taken into consideration during electric field applications is the direction of the electric field applied. In this study, vertical and horizontal application of electric fields increased MDA and SOD levels compared to the controls (Fig. 1–4). Both vertical and horizontal fields of 1.8, 1.5, 1.35 and 1 kV/m electric fields increased SOD and MDA levels significantly more than 0.8, 0.6 and 0.3 kV/m electric fields. In an earlier investigation into the effects of vertical and horizontal electric field on protein synthesis in liver, kidney, and lung tissues we observed that vertical electric field was more effective than horizontal field (Güler and Seyhan Atalay 1996; Güler et al. 1996). Similarly, Marino found that vertical electric field is more effective than horizontal field (Marino et al. 1983). In a research done in plant roots, it has been noted that vertical electric field is more effective on the growth rate of roots (Roberston et al. 1981; Miller et al. 1983). Also in this study, vertical electric fields were found to be more effective than horizontal fields, yet the differences were not statistically significant ($p > 0.05$).

The effect of 50 Hz electric field on TBARS and SOD levels was investigated for different application periods (Figs. 5–6). The results indicate that for plasma and for liver, lung, and kidney, the threshold exposure periods for 50 Hz 1.35 kV/m

electric fields are 1 day and 3 days, respectively. In this study, the elevations of both TBARS and SOD levels increased with increasing exposure time and reached maximum after ten days of exposure. Other researchers have reported that longer periods of electric field exposure had greater effects on living organisms while Benov and his colleagues also showed that an increase in application period resulted in an increase in TBARS levels (Marino et al. 1986; Cossarizza et al. 1993; Margonato et al. 1993; Benov et al. 1994).

In parallel studies, we have found that electric field-induced variations in parameters like hydroxyproline, blood gamma glutamyl transferase, cholesterol, triglyceride, urea, uric acid, phosphate, glucose, creatinine, calcium, chlorine, aspartate aminotransferase, alkaline phosphatase, alanine aminotransferase, lactate dehydrogenase, total blood protein, albumin were dependent on the period of application of the electric field (Güler and Seyhan Atalay 1997).

In this study, exposure to both static and 50 Hz electric fields led to an increase in TBARS levels in plasma, liver, lung, and kidney tissues of guinea pigs. We hypothesized in an earlier study that, as a result of energy transfer in the tissue in an electric field, molecular O_2 is transformed to free radicals and as a result of increased levels of $O_2^{\cdot-}$ radicals, TBARS levels in those tissues have increased (Güler et al. 1996). The increased TBARS levels in this study can be attributed to the same factor. We also found in this study that an increase in TBARS level is accompanied by an increase in SOD activity.

The increase in SOD activity may be regarded as an indicator of increased ROS production occurring during the exposure period and may reflect the pathophysiological process of the exposure. There are several reports indicating that free radicals are involved in EMF-induced tissue injury (Chan 2001; Irmak et al. 2002). In the investigation of Irmak and co-workers, increase in TBARS level was also accompanied by an increase in SOD activity, yet the increase in TBARS level was not statistically significant. The underlying mechanisms are unclear, though we may argue that enhanced production of superoxide radicals might increase SOD synthesis. There are other studies stating that SOD activity increases with TBARS level (Yeh et al. 1997).

Similar to this study, many experimental studies reported on biological and health effects of exposure to electric fields in different directions, strengths and periods in the range of several kV/ms, to which people near power lines are usually exposed. Due to overpopulation and industrial and technological development, there is an increased need for electric energy, transition lines and high voltage power lines. Power lines are important in the aspects of adverse biological and health effects on living organisms due to generation of high-level electric fields (van Wijngaarden et al. 2000; Kaune et al. 2002; Qiu et al. 2004).

The minimum electric field level of a 750 kV power line is 1 kV/m at cord maximal height (20 m), but is as much as 12 kV/m at cord minimal height (13 m) (Grandolfo and Vecchia 1985). Although international guidelines about power lines require that power lines should be constructed out of residential areas, due to uncontrolled urban sprawl in developing countries, power lines passing through

residential areas are a great risk for human health. The results of this study indicate that electric fields in the range of several kV/m, to which we are unconsciously exposed in our daily life, are effective on SOD activity and TBARS level.

References

Barriviera M. L., Louro S. R., Wajnberg E., Hasson-Voloch A. (2001): Denervation alters protein-lipid interactions in membrane fractions from electrocytes of *Electrophorus electrius* (L.). Biophys. Chem. **91**, 93—104

Bediz C. S., Baltaci A. K., Mogulkoc R., Öztekin E. (2006): Zinc supplementation ameliorates electromagnetic field-induced lipid peroxidation in the rat brain. Tohoku J. Exp. Med. **208**, 133—140

Benov L. C., Antonov P. A., Ribarov S. R. (1994): Oxidative damage of the membrane lipids after electroporation. Gen. Physiol. Biophys. **13**, 85—97

Beuge J. A., Aust S. D. (1978): Microsomal lipid peroxidation. Methods Enzymol. **52**, 302—310

Blumenthal N. C., Ricci J., Breger L., Zychlinsky A., Solomon H., Chen G. G., Kuznetsov D., Dorfman R. (1997): Effects of low-intensity AC and/or DC electromagnetic fields on cell attachment and induction of apoptosis. Bioelectromagnetics **18**, 264—272

Cevc G., Svetina S., Zeks B. (1981): Electrostatic potential of bilayer lipid membranes with the structural surface charge smeared perpendicular to the membrane-solution interface. An extension of the Gouy–Chapman diffuse double layer theory. J. Phys. Chem. **85**, 1762—1767

Chan P. H. (2001): Reactive oxygen radicals in signaling and damage in the ischemic brain. J. Cereb. Blood Flow Metab. **21**, 2—14

Cossarizza A., Capri M., Salvioli S., Monti D., Franceschi C., Bersani F., Cadossi R., Zucchini P., Angioni S., Petraglia F., Scarfi M. R. (1993): Electromagnetic fields affect cell proliferation and cytokine production in human cells. In: Electricity and Magnetism in Biology and Medicine (Ed. M. Blank), pp. 640—642, San Francisco Press

Desideri A., Falconi M., Polticelli F., Bolognesi M., Djinovic K., Rotilio G. (1992): Evolutionary conservativeness of electric field in the Cu,Zn superoxide dismutase active site. Evidence for co-ordinated mutation of charged amino acid residues. J. Mol. Biol. **223**, 337—342

Durak I., Yurtarslani Z., Canbolat O., Akyol O. (1993): A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. Clin. Chim. Acta **214**, 103—104

Engelmann W., Hellrung W., Johnsson A. (1996): Circadian locomotor activity of musca flies: recording method and effects of 10 Hz square-wave electric fields. Bioelectromagnetics **17**, 100—110

Grandolfo M., Vecchia P. (1985): Natural and man-made environmental exposures to static and ELF electromagnetic fields. In: Biological Effects and Dosimetry of Static and ELF Electromagnetic Fields (Eds. M. Grandolfo, S. M. Michaelson and A. Rindi), pp. 49—70, Plenum Press, New York and London

Güler G., Seyhan Atalay N. (1996): Changes in hydroxyproline levels in electric field tissue interaction. Indian J. Biochem. Biophys. **33**, 531—533

Güler G., Seyhan Atalay N. (1997): Functional enzymes of liver, total blood protein and albumin levels under electric fields. Med. Biol. Eng. Comput. (Suppl. 1), **45**

Güler G., Seyhan Atalay N., Özoğul C., Erdoğan D. (1996): Biochemical and structural approach to collagen synthesis under electric fields. Gen. Physiol. Biophys. **15**, 429—440

Güler G., Hardalac F., Aricioglu A. (2005): Examination of electric field effects on tissues by using back propagation neural network. J. Med. Syst. **29**, 679—708

Hallivell B., Gutteridge J. M. (2000): Free Radicals in Biology and Medicine. Oxford University Press, New York

Harakawa S., Inoue N., Hori T., Tochio K., Kariya T., Takahashi K., Doge F., Suzuki H., Nagasawa H. (2005): Effects of 50 Hz electric field on plasma lipid peroxide level and antioxidant activity in rats. Bioelectromagnetics **26**, 589—594

Irmak M. K., Fadillioğlu E., Güleç M., Erdoğan H., Yağmurca M., Akyol Ö. (2002): Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. Cell Biochem. Funct. **20**, 279—283

Ivancsits S., Diem E., Jahn O., Rudiger H. W. (2003): Intermittent extremely low frequency electromagnetic fields cause DNA damage in a dose-dependent way. Int. Arch. Occup. Environ. Health **76**, 431—436

Kaune W. T., Dovan T., Kavet R. I., Savitz D. A., Neutra R. R. (2002): Study of high- and low- current-configuration homes from the 1988 Denver Childhood Cancer Study. Bioelectromagnetics **23**, 177—188

Kovacs E., Dinu A., Savopol T. (1992): The interaction of the protoreceptor cells with the constant electrical field. In: Charge and Field Effects in Biosystems-3 (Eds. M. J. Allen, S. F. Cleary, A. E. Sowers and D. D. Shillady), pp. 341—347, Birkhäuser, Boston–Basel–Berlin

Lee J., Stormshak F., Thompson J., Thinesen P., Painter L., Olenchek B., Hess D., Forbes R. (1993): Endocrine responses of ewe lambs exposed to 60-Hz electric and magnetic fields from 500 kV transmission line. In: Electricity and Magnetism in Biology and Medicine (Ed. M. Blank), pp. 401—404, San Francisco Press

Lee B. C., Johng H. M., Lim J. K., Jeong J. H., Baik K. Y., Nam T. J., Lee J. H., Kim J., Sohn U. D., Yoon G., Shin S., Soh K. S. (2004): Effects of extremely low frequency magnetic field on the antioxidant defense system in mouse brain: a chemiluminescence study. J. Photochem. Photobiol. B., Biol. **73**, 43—48

Lowry O. H., Rosebrough N. I., Farr A. L., Randall R. J. (1951): Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**, 265—275

Margonato V., Veicsteinas A., Conti R., Nicolini P., Cerretelli P. (1993): Biologic effects of prolonged exposure to ELF electromagnetic fields in rats. I. 50 Hz electric fields. Bioelectromagnetics **14**, 479—493

Marino A. A., Berger T. J., Mitchell J. T., Duhacek B. A., Becker R. (1983): Electric field effects in selected biologic systems. Ann. N. Y. Acad. Sci. **405**, 436—444

Marino A. A., Morris D. M., Arnold T. (1986): Electrical treatment of lewis lung carcinoma in mice. J. Surg. Res. **41**, 198—201

Miller M. W., Dooley D. A., Cox C., Carstensen E. L. (1983): On the mechanism of 60-Hz electric field ınduced effects in *Pisum sativum* L. roots: vertical field exposures. Radiat. Environ. Biophys. **22**, 293—302

Moos W. S. (1964): A preliminary report on the effects of electric fields on mice. Aerosp. Med. **35**, 374—377

Moustafa Y. M., Moustafa R. M., Belacy A., Abou-El-Ela S. H., Ali F. M. (2001): Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. J. Pharm. Biomed. Anal. **26**, 605—608

Oury T. D., Day B. J., Crapo J. D. (1996): Extracellular superoxide dismutase in vessels and airways of humans and baboons. Free Radic. Biol. Med. **20**, 957—965

Ozguner F., Oktem F., Ayata A., Koyu A., Yilmaz H. R. (2005): A novel antioxidant agent caffeic acid phenethyl ester prevents long-term mobile phone exposure-induced renal impairment in rat. Prognostic value of malondialdehyde, N-acetyl-β-D-glucosaminidase and nitric oxide determination. Mol. Cell. Biochem. **277**, 73—80

Qiu C., Fratiglioni L., Karp A., Winblad B., Bellander T. (2004): Occupational exposure to electromagnetic fields and risk of Alzheimer's disease. Epidemiology **15**, 687—694

Repacholi M. H., Greenebaum B. (1999): Interaction of static and extremely low frequency electric and magnetic fields with living systems: health effects and research needs. Bioelectromagnetics **20**, 133—160

Robertson D., Miller M. W., Cox C., Davis H. T. (1981): Inhibition and recovery of growth processes in roots of *Pisum sativum* L. exposed to 60-Hz electric fields. Bioelectromagnetics **2**, 329—340

Scaiano J. C., Mohtat N., Cozens F. L., McLean J., Thansandote A. (1994): Application of the radical pair mechanism to free radicals in organized systems: can the effects of 60 Hz be predicted from studies under static fields? Bioelectromagnetics **15**, 549—554

Stevens R. G. (2004): Electromagnetic fields and free radicals. Environ. Health Perspect. **112**, 687—694

Sun Y., Oberley L. W., Li Y. (1988): A simple method for clinical assay of superoxide dismutase. Clin. Chem. **34**, 497—500

van Wijngaarden E., Savitz D. A., Kleckner R. C., Cai J., Loomis D. (2000): Exposure to electromagnetic fields and suicide among electric utility workers: a nested case-control study. Occup. Environ. Med. **57**, 258—263

Wilson B. W., Chess E. K., Anderson L. E. (1986): 60 Hz electric field effects on pineal melatonin rhythms: time course of onset and recovery. Bioelectromagnetics **7**, 239—242

Yeh S. L., Chang K. Y., Huang P. C., Chen W. J. (1997): Effects of n-3 and n-6 fatty acids on plasma eicosanoids and liver antioxidant enzymes in rats receiving total parenteral nutrition. Nutrition **13**, 32—36

Yokus B. Çakir D. U., Akdag M. Z., Sert C., Mete N. (2005): Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields. Free Radic. Res. **39**, 317—323

Yoshioka T., Kawada K., Shimada T., Mori M. (1979): Lipid peroxidation in maternal and cord blood and protective mechanisms against activated oxygen toxicity in blood. Am. J. Obstet. Gynecol. **135**, 372—376