The effects of exposure to electromagnetic field on rat myocardium

Amac Kiray, Hamid Tayefi, Muge Kiray, Husnu Alper Bagriyanik, Cetin Pekcetin, Bekir Ugur Ergur and Candan Ozogul

Toxicol Ind Health 2013 29: 418 originally published online 9 February 2012
DOI: 10.1177/0748233711434957

Toxicology and Industrial Health
29(5) 418–425

DOI: 10.1177/0748233711434957
tih.sagepub.com

The effects of exposure to electromagnetic field on rat myocardium

Amac Kiray[1], Hamid Tayefi[2], Muge Kiray[3], Husnu Alper Bagriyanik[4], Cetin Pekcetin[4], Bekir Ugur Ergur[4] and Candan Ozogul[5]

Abstract

Exposure to electromagnetic fields (EMFs) causes increased adverse effects on biological systems. The aim of this study was to investigate the effects of EMF on heart tissue by biochemical and histomorphological evaluations in EMF-exposed adult rats. In this study, 28 male Wistar rats weighing 200–250 g were used. The rats were divided into two groups: sham group ($n = 14$) and EMF group ($n = 14$). Rats in sham group were exposed to same conditions as the EMF group except the exposure to EMF. Rats in EMF group were exposed to a 50-Hz EMF of 3 mT for 4 h/day and 7 days/week for 2 months. After 2 months of exposure, rats were killed; the hearts were excised and evaluated. Determination of oxidative stress parameters was performed spectrophotometrically. To detect apoptotic cells, terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) staining and caspase-3 immunohistochemistry were performed. In EMF-exposed group, levels of lipid peroxidation significantly increased and activities of superoxide dismutase and glutathione peroxidase decreased compared with sham group. The number of TUNEL-positive cells and caspase-3 immunoreactivity increased in EMF-exposed rats compared with sham. Under electron microscopy, there were mitochondrial degeneration, reduction in myofibrils, dilated sarcoplasmic reticulum and perinuclear vacuolization in EMF-exposed rats. In conclusion, the results show that the exposure to EMF causes oxidative stress, apoptosis and morphologic damage in myocardium of adult rats. The results of our study indicate that EMF-related changes in rat myocardium could be the result of increased oxidative stress. Further studies are needed to demonstrate whether the exposure to EMF can induce adverse effects on myocardium.

Keywords

Electromagnetic field, myocardium, oxidative stress, apoptosis, ultrastructure

Introduction

Electromagnetic fields (EMFs) have biological effects depending on its frequency and exposure time. Due to increasing distribution and widespread use of the electrical machines and electronic equipment, today everyone is exposed to EMF. EMF of 50 Hz was generated from domestic electric distribution systems. Each electrical home appliance from oven to television is an EMF source that may affect human health. EMF affects biological systems by prolonging the life of free radicals in the systems. Free oxygen radicals cause oxidative damage to DNA, impairment of protein function and peroxidation of lipids. Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to the oxidation of

[1]Department of Anatomy, Dokuz Eylul University Medical School, Turkey
[2]Department of Anatomy, Tehran University Medical School, Iran
[3]Department of Physiology, Dokuz Eylul University Medical School, Turkey
[4]Department of Histology and Embryology, Dokuz Eylul University Medical School, Turkey
[5]Department of Histology and Embryology, Gazi University Medical School, Turkey

Corresponding author:
Muge Kiray, Department of Physiology, Dokuz Eylul University Medical School, Balcova, Izmir, Turkey.
Email: muge.kiray@deu.edu.tr

polyunsaturated fatty acids and thus causing oxidative stress (Goraca et al., 2010; Meral et al., 2007). EMF could cause oxidative DNA damage and increases lipid peroxidation in tissues (Yokus et al., 2005; Yurekli et al., 2006). The reports demonstrated that oxidative damage has been implicated in myocardial injuries (Annapurna et al., 2008). Ozguner et al. (2005a) reported an increase in lipid peroxidation in the heart of rat following exposure to mobile phone device. The intracellular enzymatic defense against free radicals involves superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (Guler et al., 2007). There are several reports which indicate that the exposure to EMF reduces antioxidant enzyme activities in the rat tissues (Elhag et al., 2007; Ozguner et al., 2005b).

The cellular damage induced by oxidative stress may trigger the process of apoptosis. Free oxygen radicals may participate in the initiation of apoptotic or necrotic cell death. EMF can induce apoptosis *in vivo* and *in vitro*, but its *in vivo* effects in myocardium are unclear (Jajte et al., 2002; Kaszuba-Zwoińska et al., 2005).

Several structural changes may occur after myocardial damage. Myocardial changes could be showed by light or electron microscopic evaluation. In our previous study, we showed that EMF caused myocardial damage in rat pups (Tayefi et al., 2010). In this study, we aimed to investigate the effects of exposure to EMF on the hearts of adult rats by biochemical and histomorphological evaluations. For biochemical evaluation, levels of MDA were assessed as a marker of lipid peroxidation and the activities of SOD and GPx were determined to evaluate the antioxidant status in the hearts of rat. Light and electron microscopic assessments were processed for histological evaluation.

Methods

Animals and experimental design

All experimental protocols were approved by the Ethics Committee of Animal Care and Experimentation of the University of Dokuz Eylul, Turkey (permit no: 79-2005). A total of 28 male Wistar rats weighing 200–250 g were used in this study. They were housed in polycarbonate cages with food and water *ad libitum*. The rats were divided into two groups; sham group (EMF(−), $n = 14$) and EMF group (EMF(+), $n = 14$). Rats in the sham group were exposed to same conditions as the EMF group except the exposure to EMF. Rats in the EMF group were exposed to a 50-Hz EMF of 3 mT for 4 h/day and 7 days/week for 2 months. After 2 months of exposure, rats were killed; the hearts were excised and evaluated.

Magnetic field exposure system

Exposure to EMF was through a device that was used previously in our laboratory (Tayefi et al., 2010). EMF of 3 mT was produced by a pair of Helmholtz coils (95 cm in diameter), each with 320 turns of 2.5 mm copper wire mounted on a wooden frame. The distance between the coils was 33 cm. Coils were connected in series to a generator delivering an AC current (Figure 1). The output current was 6.43 A at 50 Hz. The magnetic field intensity was measured by a digital teslameter (FW Bell, 5170, Pacific Scientific-OECO, Milwaukie, OR, USA). The teslameter accuracy was $\pm 2\%$ for AC.

Biochemical estimations

The animals were killed after the last exposure and the heart tissue samples obtained from left ventricles were taken for light and electron microscopic assessment and for biochemical estimations. Determination of MDA levels and antioxidant enzyme activities were performed spectrophotometrically. The Bioxytech MDA-586 (Oxis International, Foster City, CA, USA) assay for MDA, the Bioxytech SD-525 (Oxis International, Foster City, CA, USA) assay for SOD activity and the Bioxytech GPx-340 (Oxis International, Foster City, CA, USA) assay for GPx activity were performed per the kit protocol. All enzyme activities were assayed with a Hach Lange DR5000 UV spectrophotometer.

Histomorphological examination

The dissected hearts of rat were immediately placed in 10% formalin in phosphate buffer overnight, processed by routine histological methods and embedded in paraffin blocks. Paraffin blocks were placed in Leica RM2255 rotary microtome (Wetzlar, Germany) and sections of 5 μm thickness were obtained. Sections were stained for terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL), and caspase-3 immunohistochemistry was performed. The images were analyzed using a computer-assisted image analyzer system consisting of a microscope (Olympus BH-2, Tokyo, Japan) equipped with a high-resolution video camera (JVC TK-890E, Japan).

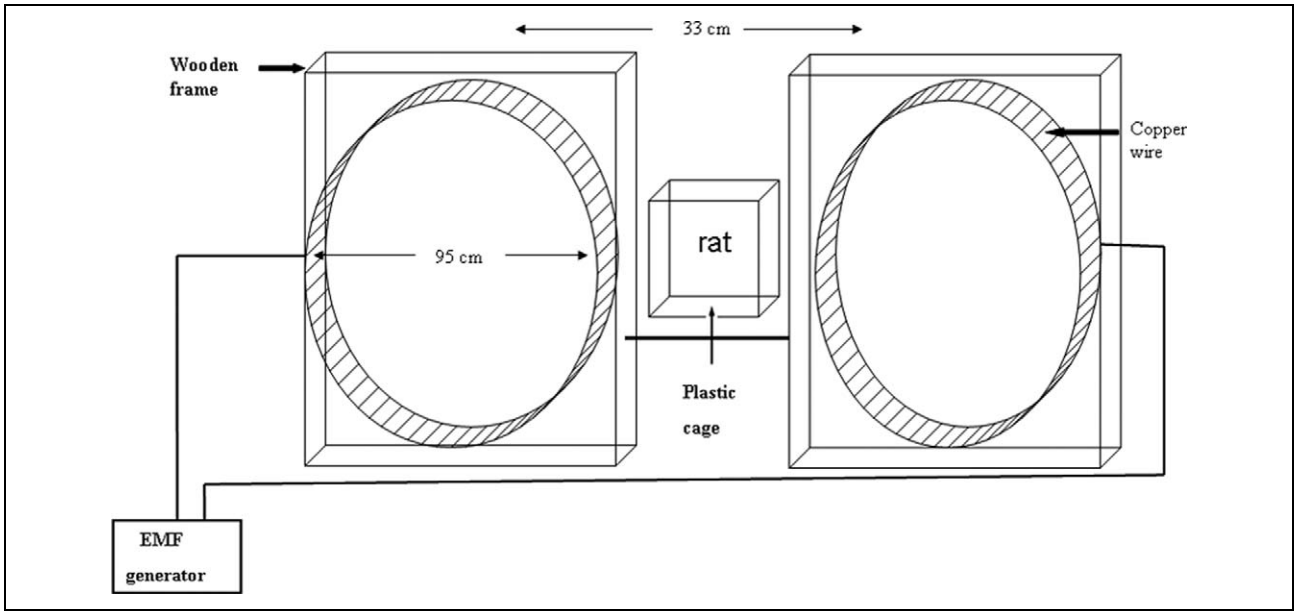

Figure 1. Schematic representation of the exposure system.

For ultrastructural investigations, the left ventricle pieces were placed in 2.5% glutaraldehyde for 24 h for fixation. The tissue was postfixed with osmium tetroxide, dehydrated in a graded series of alcohol and then embedded in Araldite® CY212. The thin (60–90 nm) sections were obtained with ultramicrotome (Leica) and stained with uranyl acetate and lead citrate, examined on a transmission electron microscope (Carl Zeiss Libra 120, Jena, Germany) and digitally photographed.

TUNEL method

TUNEL staining was performed using an In Situ Cell Death Detection Kit® (Roche, Mannheim, Germany), according to the manufacturer's protocol. Briefly, the sections were deparaffinized, hydrated by successive series of alcohol, washed in distilled water followed by phosphate-buffered saline and deproteinized by proteinase K (20 µg/ml) for 30 min at 37°C. Then the sections were rinsed and incubated in the TUNEL reaction mixture. The sections were rinsed and visualized using converter peroxidase (POD) with 0.02% 3,3′-diaminobenzidine (DAB). The sections were counterstained with hematoxylin. Ten fields were randomly chosen for each slide and a total of 100 cells per field were counted (×20 objective).The apoptotic index (percentage of apoptotic nuclei) was calculated as apoptotic nuclei/total nuclei counted × 100%. All counting procedures were performed blindly.

Caspase-3 immunohistochemistry

For visualization of the caspase-3 expression, caspase-3 immunohistochemistry was performed using an anti-caspase-3 antibody. The sections were incubated overnight with anti-caspase-3 antibody (1:100; Neomarkers, Fremont, California, USA) and then for another 30 min with the biotinylated mouse secondary antibody. The bound secondary antibody was then amplified with Vector Elite ABC kit® (Vectastain, Vector Laboratories, Burlingame, California, USA). The antibody avidin–biotin–peroxidase complexes were visualized using 0.02% DAB. The sections were finally mounted onto lysine-coated slides. A semiquantitative immunolabeling scale from 1 to 4 were graded as; 1: *none*, 2: *mild*, 3: *moderate* and 4: *strong* (Müller-Krebs et al., 2008).

Statistical analysis

Results are presented as means ± SEM. Statistical analyses were carried out using SPSS software (SPSS 15.0 for Windows). All the data were analyzed by Mann-Whitney test. $p < 0.05$ was considered statistically significant.

Results

Biochemical analyses

Figure 2 shows the levels of MDA in the hearts of rats. Level of MDA in sham group was 2.99 ± 0.37. In the

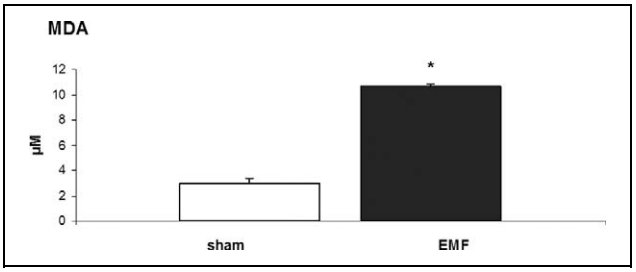

Figure 2. Effects of exposure to electromagnetic field on the levels of malondialdehyde in the hearts of rat. Data are mean ± SEM. *$p < 0.05$ compared with the sham group.

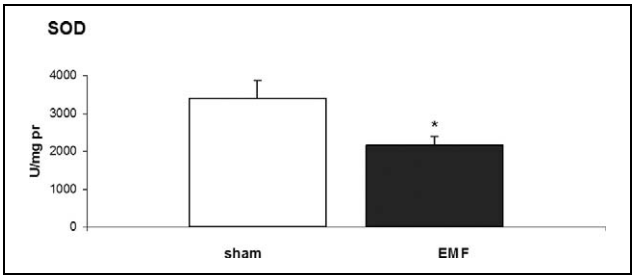

Figure 3. Effects of exposure to electromagnetic field on the activity of superoxide dismutase in the hearts of rat. Data are mean ± SEM. *$p < 0.05$ compared with the sham group.

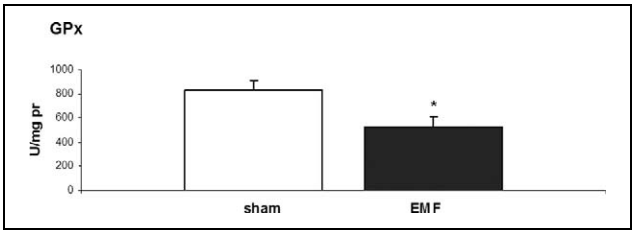

Figure 4. Effects of exposure to electromagnetic field on the activity of glutathione peroxidase in the hearts of rat. Data are mean ± SEM. *$p < 0.05$ compared with the sham group.

EMF-exposed group, levels of MDA in tissue were significantly increased compared with the sham group (10.64 ± 0.18, $p = 0.02$). The results of activities of SOD enzymeare shown in Figure 3. Activities of SOD enzyme decreased significantly in EMF-exposed group compared with the sham group (2159.82 ± 222.5 and 3407.17 ± 472.7, respectively; $p = 0.025$). The results of activities of GPx enzyme are shown in Figure 4. Activities of GPx enzyme decreased significantly in EMF-exposed group compared with the sham group (532.42 ± 74.7 and 827.1 ± 81.8, respectively; $p = 0.003$).

Histological examination

The present study shows that EMF-exposure enhanced apoptotic cell death in the rat myocardium. Sham rats showed fewer TUNEL-positive cells in myocardium. There were more TUNEL-positive cells in EMF-exposed group, as seen in Figure 5. Quantification and statistical analysis of the TUNEL staining showed that the number of TUNEL-positive cells increased significantly in EMF-exposed rats compared with the sham rats (16.35 ± 1.12 vs 7.28 ± 0.49, $p = 0.002$).

Apoptotic feature was further confirmed by caspase-3 immunohistochemistry. Immunohistochemical evaluation based on the intensity of caspase-3 immunoreactivity in the rat myocardium is shown in Figure 6. Representative photographs of caspase-3 expression show that reactivity of caspase-3 was commonly observed in the rat myocardium in EMF-exposed rats compared with sham rats. According to the scoring system, the intensity of caspase-3 immunoreactivity in the EMF-exposed group was found to have increased when compared with the sham group (3.14 ± 0.26 vs 1.42 ± 0.20, $p = 0.002$).

In electron microscopic examination, no ultrastructural changes were observed in the heart specimens of sham group; cellular and mitochondrial structures were normal (Figure 7(a1) to (a2)). In the EMF-exposed group specimens, there were dilated sarcoplasmic reticulum (Figure 7(b1) to (b2)) and mitochondrial degeneration (Figure 7(c)). Most of the myofibrils disappeared and were fragmented (Figure 7(b)), and perivascular vacuoles were formed (Figure 7(d)).

Discussion

In this study, we demonstrated that EMF causes oxidative damage by increasing the levels of MDA and suppressing the activities of SOD and GPx in the heart tissue of adult rats. Additionally, the findings of this study indicated that exposure to EMF induced apoptosis and ultrastructural changes in the heart tissue.

Our results indicate that exposure to 50-Hz EMF of 3 mT for 4 h/day and 7 days/week for 2 months induces lipid peroxidation in myocardial tissue of rats. Lipid peroxidation is one of the determinants of oxidative stress induced by free oxygen radicals. Our findings indicate that EMF caused increased generation of free oxygen radical in the hearts of rat. Experimental studies suggest that lipid peroxidation resulting from free oxygen radicals contributes to the EMF-induced oxidative damage (Hashish et al., 2008;

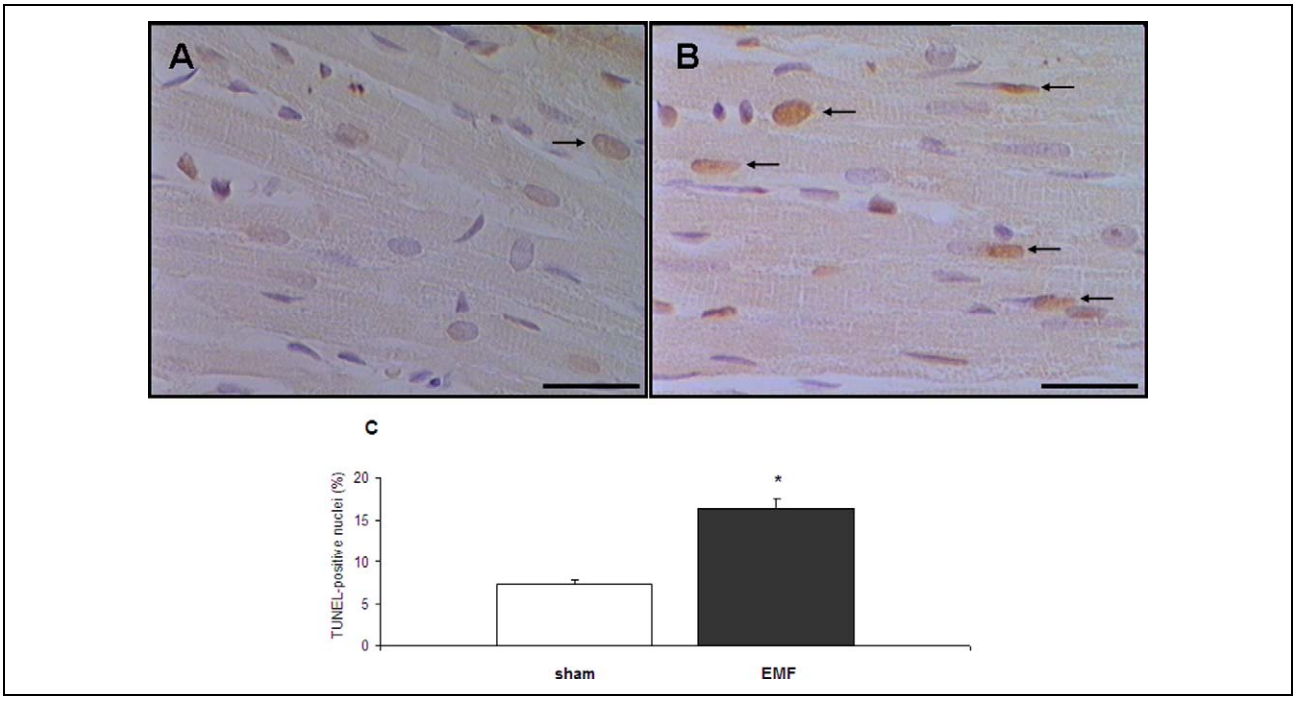

Figure 5. Upper: The effect of exposure to EMF on apoptosis in the hearts of rat. Representative photomicrographs of TUNEL-positive cells (arrows). (a) and (b), sections of sham and EMF groups, respectively (scale bar = 30 μm). Lower, (c): Quantitative analysis of TUNEL-positive cells in the heart of rat. Data are mean ± SEM. * $p < 0.05$ compared with the sham group. EMF: electromagnetic field; TUNEL: terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling.

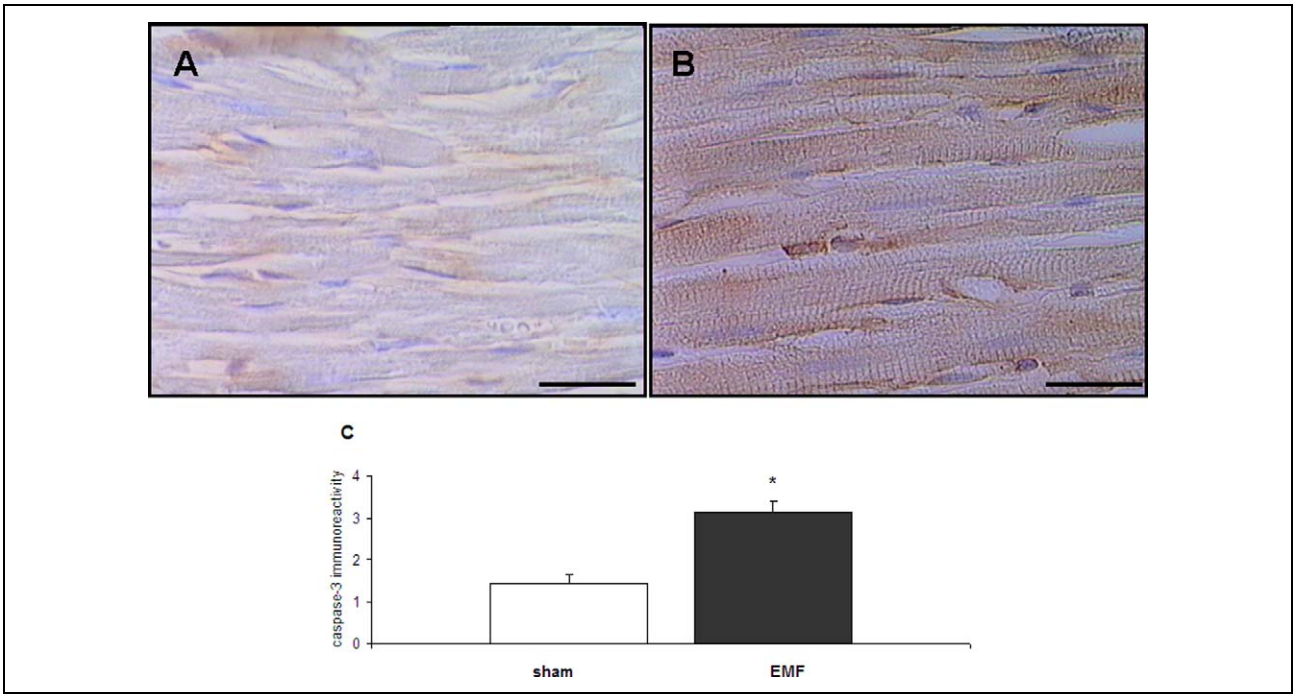

Figure 6. Upper: The effect of exposure to EMF on caspase-3 immunoreactivity in the hearts of rat. Photomicrographs of (a) and (b) show sections of sham and EMF groups, respectively (scale bar = 30 μm). Lower, (c): Semiquantitative analysis of caspase-3 immunoreactive cells in the heart of rat. Data are mean ± SEM. *$p < 0.05$ compared with the sham group. EMF: electromagnetic field.

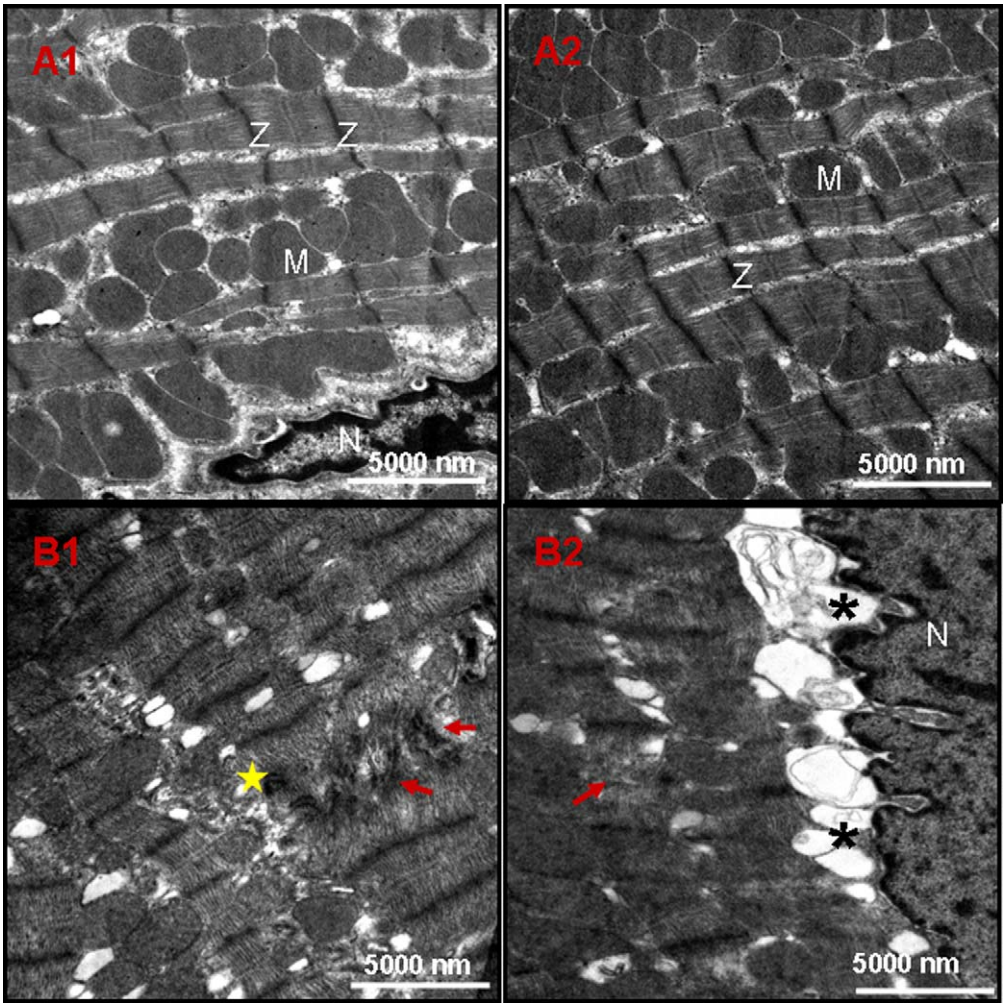

Figure 7. Electron micrographs of the left ventricular myocardium of sham ((a1) and (a2)) and EMF-exposed groups ((b1) and (b2)). (a1) and (a2): Normal myocytes, regularly arranged myofilaments, mitochondria (M), nucleus (N) and Z-lines (Z). (b1): degeneration and fragmentation of myofibrils (red arrows); loss of cristae and swelling in mitochondria (*). (b2): fragmentation of myofibrils (red arrow) and perinuclear vacuoles (*). EMF: electromagnetic field.

Seyhan and Canseven, 2006). The organism has some protective defense enzymes and repair systems against oxidative damage. These defense systems include enzymes of SOD and GPx. SOD catalyzes the dismutation of superoxide free radical to dioxygen and to the less-reactive species oxygen peroxide. GPx decompose peroxides to water. These enzymatic antioxidants provide protection for biological systems (Valko et al., 2006). In this study, we assessed the activities of SOD and GPx in myocardial tissue of adult rats. The activities of antioxidant enzyme were found to be significantly decreased in EMF-exposed rats. Several studies indicated that EMF alters the generation of free radical and antioxidative defenses. Martínez-Sámano et al. (2010) reported that acute exposure to EMF decreases the activity of SOD but does not induce lipid peroxidation in the heart tissue. Ozguner et al. (2005a) demonstrated that exposure to mobile phone device increased lipid peroxidation and decreased the activities of antioxidant enzyme in rat myocardium. Goraca et al. (2010) reported that exposure to EMF of 40 Hz, 7 mT, 60 min/day for 2 weeks resulted in free radical-induced oxidative stress in the heart tissue. The results may vary depending on the duration and type of exposure, but the effect of subchronic exposure to EMF in the heart tissue of adult rats has not yet been well established.

At the end of the experiment, the cardiomyocyte apoptosis was detected by TUNEL method and caspase-3 immunohistochemistry. TUNEL method is widely used to detect apoptotic cell death in tissue sections. Caspase-3 immunohistochemistry is also

used to identify the cells undergoing apoptosis. Caspase-3 is a member of a family of intracellular apoptosis-associated proteases that are involved in the initiation and execution of apoptosis. Once caspases are activated, they cleave a large number of substrate proteins within the cell, thereby allowing the structural disaggregation of the cell, seen during the late stages of apoptosis, to proceed. Their activation occurs in response to a variety of proapoptotic stimuli and constitutes a final common pathway in the cell death process. The detection of caspase-3 is a valuable tool in histological labeling of dying cells (Stadelmann and Lassmann, 2000). Our results showed that exposure to EMF induces apoptotic cell death in adult rats. The effect of EMF on cardiomyocyte apoptosis is associated with marked increase in MDA. Free radicals are behind the mechanism of cell death caused by exposure to mT EMF. Cardiomyocyte apoptosis is associated with several cardiac diseases. Although increased activity of apoptosis in the heart tissue exposed to EMF has been reported *in vitro*, the *in vivo* effect of subchronic exposure to EMF in rat myocardium has not been previously investigated (Gottwald et al., 2007).

In this study, the histological evidence of EMF-related myocardial tissue injury was shown with electron microscopy. The ultrastructural modifications were assessed to investigate the adverse effects of subchronic exposure to EMF in the heart tissue. Under electron microscopy, mitochondrial swelling, reduction in myofibrils, expanded sarcoplasmic reticulum and perinuclear vacuolization were observed. The histological alterations in myocardial ultrastructure were shown in various myocardial damage models. He et al. (2007) demonstrated that hearts of rats with diabetes show mitochondrial disruption and myofibrillary disarrangement. Clements et al. (2010) indicated pathological changes in myofibrils, mitochondria and sarcoplasmic reticulum in cardiotoxicity of drug. In our study, we demonstrated EMF-induced myocardial morphological changes in the ultrastructure of adult rats.

In conclusion, the results show that subchronic exposure to EMF causes apoptosis and morphologic changes in myocardium of adult rats. Exposure to EMF induces oxidative stress by reducing the activity of SOD and GPx parallel to increasing lipid peroxidation. We have provided support for parameters that examine EMF-related impairment in the myocardium of rats caused by exposure to EMF. Further studies with different exposure times and type of EMF are needed to demonstrate whether the exposure to EMF can induce adverse effects on rat myocardium.

Acknowledgements

The authors thank Ozcan Ustun and Sedef Menku for their excellent technical assistance.

Conflict of interest

The authors declared no conflicts of interest

Funding

This work was supported by Dokuz Eylul University Research Foundation (grant no: 2005.KB.SAG.012).

References

Annapurna A, Challa SR, Prakash GJ and Viswanath RK (2008) Therapeutic potential of sulindac against ischemia-reperfusion-induced myocardial infarction in diabetic and nondiabetic rats. *Experimental and Clinical Cardiology* 13(2): 66–70.

Clements P, Brady S, York M, et al. (2010) Time course characterization of serum cardiac troponins, heart fatty acid-binding protein, and morphologic findings with isoproterenol-induced myocardial injury in the rat. *Toxicologic Pathology* 38(5): 703–714.

Elhag MA, Nabil GM and Attia AM (2007) Effects of electromagnetic field produced by mobile phones on the oxidant and antioxidant status of rats. *Pakistan Journal of Biological Sciences* 10(23): 4271–4274.

Goraca A, Ciejka E and Piechota A (2010) Effects of extremely low frequency magnetic field on the parameters of oxidative stress in heart. *Journal of Physiology and Pharmacology* 61(3): 333–338.

Gottwald E, Sontag W, Lahni B and Weibezahn KF (2007) Expression of HSP72 after ELF-EMF exposure in three cell lines. *Bioelectromagnetics* 28: 509–518.

Guler G, Turkozer Z and Seyhan N (2007) Electric field effects on Guinea pig serum: the role of free radicals. *Electromagnetic Biology and Medicine* 26(3): 207–223.

Hashish AH, El-Missiry MA, Abdelkader HI and Abou-Saleh RH (2008) Assessment of biological changes of continuous whole body exposure to static magnetic field and extremely low frequency electromagnetic fields in mice. *Ecotoxicololgy and Environmental Safety* 71: 895–902.

He RX, Gu CL, Shen F and Zhang XM (2007) Changes in expression of adrenomedullin in the myocardium of streptozotocin-induced diabetic rats. *Chinese Medical Journal (English Edition)* 120(3): 187–191.

Jajte J, Grzegorczyk J, Zmyślony M and Rajkowska E (2002) Effect of 7 mT static magnetic field and iron ions on rat lymphocytes: apoptosis, necrosis and free radical processes. *Bioelectrochemistry* 57(2): 107–111.

Kaszuba-Zwoińska J, Gil K, Ziomber A, et al. (2005). Loss of interstitial cells of Cajal after pulsating electromagnetic field (PEMF) in gastrointestinal tract of the rats. *Journal of Physiology and Pharmacology* 56(3): 421–432.

Martínez-Sámano J, Torres-Durán PV, Juárez-Oropeza MA, Elías-Viñas D and Verdugo-Díaz L (2010) Effects of acute electromagnetic field exposure and movement restraint on antioxidant system in liver, heart, kidney and plasma of Wistar rats: a preliminary report. *International Journal of Radiation Biology* 86(12): 1088–1094.

Meral I, Mert H, Mert N, et al. (2007) Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. *Brain Research* 1169: 120–124.

Müller-Krebs S, Kihm LP, Zeier B, et al. (2008) Renal toxicity mediated by glucose degradation products in a rat model of advanced renal failure. *European Journal of Clinical Investigation* 38(5): 296–305.

Ozguner F, Altinbas A, Ozaydin M, et al. (2005a) Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. *Toxicology and Industrial Health* 21(9): 223–230.

Ozguner F, Oktem F, Ayata A, Koyu A and Yilmaz HR (2005b) 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-beta-D-glucosaminidase and nitric oxide determination. *Molecular and Cellular Biochemistry* 277(1–2): 73–80.

Seyhan N, Canseven AG (2006) In vivo effects of ELF MFs on collagen synthesis, free radical processes, natural antioxidant system, respiratory burst system, immune system activities, and electrolytes in the skin, plasma, spleen, lung, kidney, and brain tissues. *Electromagnetic Biology and Medicine* 25(4): 291–305.

Stadelmann C, Lassmann H (2000) Detection of apoptosis in tissue sections. *Cell and Tissue Research* 301: 19–31.

Tayefi H, Kiray A, Kiray M, et al.. (2010) The effects of prenatal and neonatal exposure to electromagnetic fields on infant rat myocardium. *Archieves of Medical Science* 6(6): 837–842.

Valko M, Rhodes CJ, Moncol J, Izakovic M and Mazur M (2006) Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chemico-Biological Interactions* 160(1): 1–40.

Yokus B, Cakir DU, Akdag MZ, Sert C and Mete N (2005) Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields. *Free Radical Research* 39(3): 317–323.

Yurekli AI, Ozkan M, Kalkan T, et al. (2006) GSM base station electromagnetic radiation and oxidative stress in rats. *Electromagnetic Biology and Medicine* 25(3): 177–188.