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The effects of prenatal long-duration exposure to 900-MHz electromagnetic field on the 21-day-old newborn male rat liver

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Background/aim: To determine what effect a 900-MHz electromagnetic field (EMF) applied in the prenatal period would have on the liver in the postnatal period.

Materials and methods: At the start of the study, adult pregnant rats were divided into two groups, control and experimental. The experimental group was exposed to a 900-MHz EMF for 1 h daily during days 13–21 of pregnancy. After birth, no procedure was performed on either mothers or pups. Male rat pups (n = 6) from the control group mothers (CGMR) and male rat pups (n = 6) from the experimental group mothers (EGMR) were sacrificed on postnatal day 21.

Results: Biochemical analyses showed that malondialdehyde and superoxide dismutase values increased and glutathione levels decreased in the EGMR pups. Marked hydropic degeneration in the parenchyma, particularly in pericentral regions, was observed in light microscopic examination of EGMR sections stained with hematoxylin and eosin. Examinations under transmission electron microscope revealed vacuolization in the mitochondria, expansion in the endoplasmic reticulum, and necrotic hepatocytes.

Conclusion: The study results show that a 900-MHz EMF applied in the prenatal period caused oxidative stress and pathological alterations in the liver in the postnatal period.

Key words: Electromagnetic field, liver, male rat, prenatal period

1. Introduction

Devices such as mobile phones, wireless internet modems, and radios and televisions, which occupy an important place in social life, produce electromagnetic fields (EMFs). Widespread use of these devices in daily life increases the intensity of exposure to EMFs on a day-to-day basis. Investigation of the effects on health of devices such as mobile phones used in close proximity to the body is attracting considerable interest from scientists. Mobile phones manufactured using the latest technology operate in a high frequency range (300–3000 MHz). This further heightens concerns regarding the effect of mobile phones on human health (1). Most Global System for Mobile Communications (GSM) operators in Europe, Asia, and Africa use a frequency of 900 MHz (2). Mobile phones for a 900-MHz GSM system and their base stations establish EMF in the range of 890–960 MHz. Studies have reported that EMF of such intensity leads to irreversible oxidative damage in the lymphoid organs of rats that have not yet reached adulthood as compared to adult rats (3).

Examination of studies on the subject shows that the results of EMF applied at different frequencies and for different durations vary in different tissues. For example, morphological changes such as an increase in cell size in lymphocytes exposed to a 1.8-GHz EMF and injury in the nucleus and organelles with long-term exposure have been reported (4). In another study, a 50-Hz EMF led to an increase in apoptosis and lipid peroxidation (LPO) in the adult rat heart (5). An EMF of 940 MHz (15 V/m, specific absorption rate [SAR] = 40 mW/kg) has been reported to lead to conformational changes in DNA structure in the thymus (6). Another study reported an increase in malondialdehyde (MDA), an indicator of oxidative stress, in the brain of mice exposed to low-frequency EMF (7).

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EMF has also been reported to lead to oxidative injury in testis and kidney tissues (8). Rises in stress-related metallothioneins and hematological changes have been observed in the kidney and liver even with subchronic application of EMF (9).

Although several studies have been performed in order to determine the effects of exposure to EMF in living tissue and organs, the amount of information available concerning the effects of prenatal exposure to EMF on developing organs and tissues is limited. Additionally, we encountered no studies in the literature investigating the effect of a 900-MHz EMF applied in the prenatal period on the liver in the postnatal period. The purpose of this study was therefore to investigate the effect of a 900-MHz EMF applied in the prenatal term on the newborn rat pup liver in the postnatal period.

2. Materials and methods

2.1. Experimental protocol

Ethical approval was obtained from the Karadeniz Technical University (KTU) Animal Care and Ethics Committee. The Sprague Dawley rats used were obtained from the KTU Faculty of Medicine Surgical Research Center (KTUSRC). Rats were housed and fed at the KTUSRC throughout the study. Rats were kept at room temperature (22 ± 2 °C) and humidity (50 ± 10%) in a controlled (12/12 h) light/dark cycle. Standard laboratory chow and tap water were provided. At the start of the study, six pregnant Sprague Dawley rats weighing 150–200 g were equally divided into a control group (CG) and experimental group (EG). No procedure was performed on the CG rats. Rats in the EG were exposed to the effect of a 900-MHz EMF for 1 h daily at the same time each day (1100–1200 hours) inside a specially manufactured Plexiglas jar on days 13–21 of pregnancy. We used an EMF application protocol already described in previous studies to expose EG pregnant rats to a 900-MHz EMF (10–13). During EMF application, the frequency on the EMF inside the jar with rats inside the jar was measured in order to determine the intensity of electrical field distribution. We calculated that pregnant EG rats were exposed to a mean electrical field intensity of 14.22 V/m inside the jar (0.54 W/m^2). The whole-body SAR was calculated as 0.027 W/kg (RadHaz SAR Equivalency Calculator Version 1.0, Richard Tell Associates, Inc., USA). No procedure was performed on mothers or pups after birth, and rat pups were left to feed naturally with their mothers. The study then continued with male rat pups obtained from the pregnant rats. On postnatal day 21, male rat pups obtained from control group mothers (CGMR, n = 6) and male rat pups obtained from experimental group mothers (EGMR, n=6) were sacrificed and their livers were removed. Half of the liver tissues were used in biochemical analyses and half in light microscopy and transmission electron microscopy (TEM) analyses.

2.2. Histopathological analyses

Histopathological evaluations were performed using light and electron microscopy. Tissue procedures for light microscopic analysis were performed at the KTU Faculty of Medicine's Department of Histology and Embryology. Sections 5 μm in thickness were taken using an automatic microtome (Leica RM 2255, Leica Instruments, Germany) from tissue fixed in paraffin after routine histological tissue procedures. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus BX-51, Japan) by a pathologist. Liver tissues of 1 mm in thickness were used for ultrastructural investigations. Liver tissue samples underwent sampling, prefixation, washing, postfixation, washing, dehydration, saturation, embedding, polymerization, ultrathin sectioning, observation, and photographic image capture for TEM analyses. Images were obtained on a JEOL 100SX TEM (JEOL Ltd., Akishima, Japan) and photographic image capture equipment (Kodak 4489, Eastman Kodak Company, USA).

2.3. Biochemical analyses

Superoxide dismutase (SOD) and catalase (CAT) enzyme activities and concentrations of glutathione (GSH) and LPO in liver tissues were determined. Measurement of SOD activity was performed as described by Sun et al. (14) and expressed as mmol min^{-1} mg^{-1} of tissue. Decomposition of H_2O_2 in the presence of CAT was observed at 240 nm, and CAT results were expressed as mmol min^{-1} mg^{-1} of tissue (15). The total amount of GSH in the tissues was measured using the method described by Sedlak and Lindsay (16), with some modifications. Absorbance was measured at 412 nm, and the GSH level in the liver was expressed as nmol/g tissue. The level of LPO in the liver tissues was determined by estimating MDA using the thiobarbituric acid test and was expressed as nmol MDA/g tissue (17).

2.4. Statistical analyses

Kruskal–Wallis analysis of variance and Mann–Whitney U-test with corrected Bonferroni test were used for statistical analysis of biochemical results using the SPSS 13.1 (SPSS Inc., USA). $P < 0.05$ was considered statistically significant. Statistical data are presented as mean ± standard deviation.

3. Results

3.1. Light microscopic observations

The H&E liver sections were observed under light microscopy. No pathological finding was observed in the CGMR liver specimens, and the parenchyma and portal areas had a normal appearance (Figures 1A and 1B). However, marked hydropic degeneration was present in

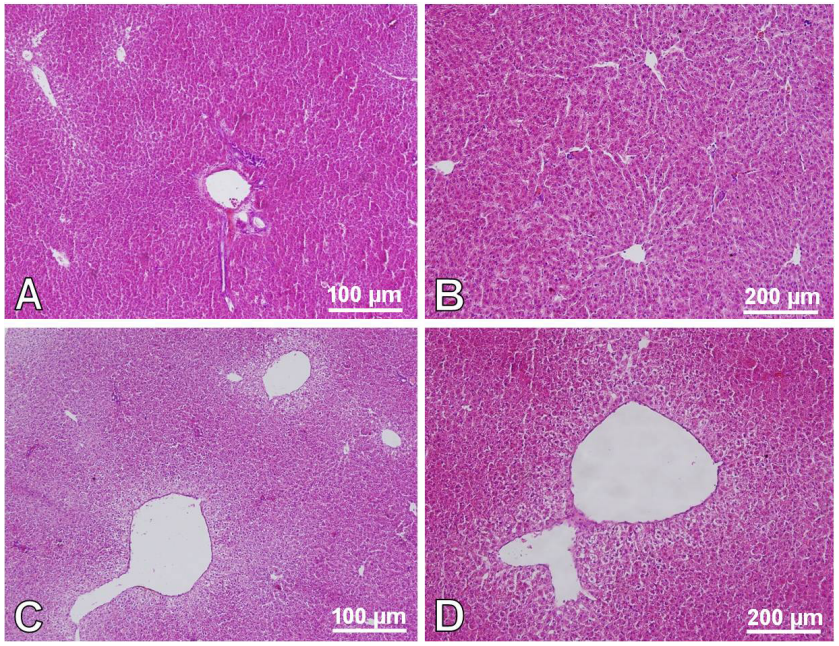

Figure 1. Light microscopic photomicrograph of liver sections of CGMR **(A** and **B)** and EGMR **(C** and **D)** (stain: hematoxylin and eosin). While there was no pathological finding in CGMR, there was significant hydropic degeneration in the hepatic parenchyma of EGMR rats, particularly in pericentral areas (zone 3). CGMR, Control group male rats; EGMR, experimental group male rats.

the hepatic parenchyma of the EGMR rats, and particularly in the pericentral areas (zone 3) (Figures 1C and 1D). No increase in inflammation or fibroblastic activity was determined in the portal areas in EGMR sections.

3.2. Electron microscopic observations

The CGMR micrographs exhibited regular hepatocytes, Disse space, and nuclear and cytoplasmic ultrastructure. Mitochondrial cristae and alpha-glycogen granules were clearly visible. Typical stellate cell processes with lipid droplet were seen in CGMR micrographs. Regular granular endoplasmic reticulum, endothelial process, and sinusoid were also observed (Figures 2A and 2B). Irregular nuclear, cytoplasmic, and sinusoid structures were seen in the EGMR micrographs (Figures 2C–2F). Necrotic hepatocytes were the most significant findings in EGMR (Figure 2C–2F). Enlarged endoplasmic reticulum and cytoplasmic and mitochondrial vacuoles were observed in hepatocytes (Figure 2C–2F). We observed Kupffer cell phagocytosis in the EGMR sections. Some hepatocytes had cytoplasmic density (Figure 2F). There were also mitotic stellate cells in the Disse space. In EGMR samples we observed activated stellate cells for fibrosis (with collagen fibrils; Figure 2E) and disrupted hepatocyte cord integrity.

3.3. Biochemical results

Biochemical analysis of liver tissues revealed significantly higher tissue MDA and SOD values in EGMR compared to those of CGMR (P = 0.08), while GSH values were lower (P = 0.08) (Table). No significant difference was determined in CAT values (P = 1.00).

4. Discussion

Studies report that exposure to toxic substances and environmental agents that may have toxic effects compromises the development of organs and systems. For example, one study reported that exposure to nonsteroidal antiinflammatory drugs in the prenatal period affected the development of the sciatic nerve (18). Another study reported that antiinflammatory drugs administered in the prenatal period reduced the number of cells in the hippocampus and led to dysfunction in the development of the nervous system (19). The effects of the EMF emitted by the mobile phones that are frequently used in social life as technology progresses have recently attracted considerable attention from researchers. For example, Sonmez et al. (20) reported that many GSM operators used a frequency of 900 MHz and that there was a marked decrease in the number of Purkinje cells in the cerebellum of adolescent female rats exposed to EMF at that frequency. Another study reported that EMF applied at a low frequency led to changes in hematological parameters (21). One study reported genotoxic effects in cells associated with chromosomal aberration and structures such as micronuclei in mature

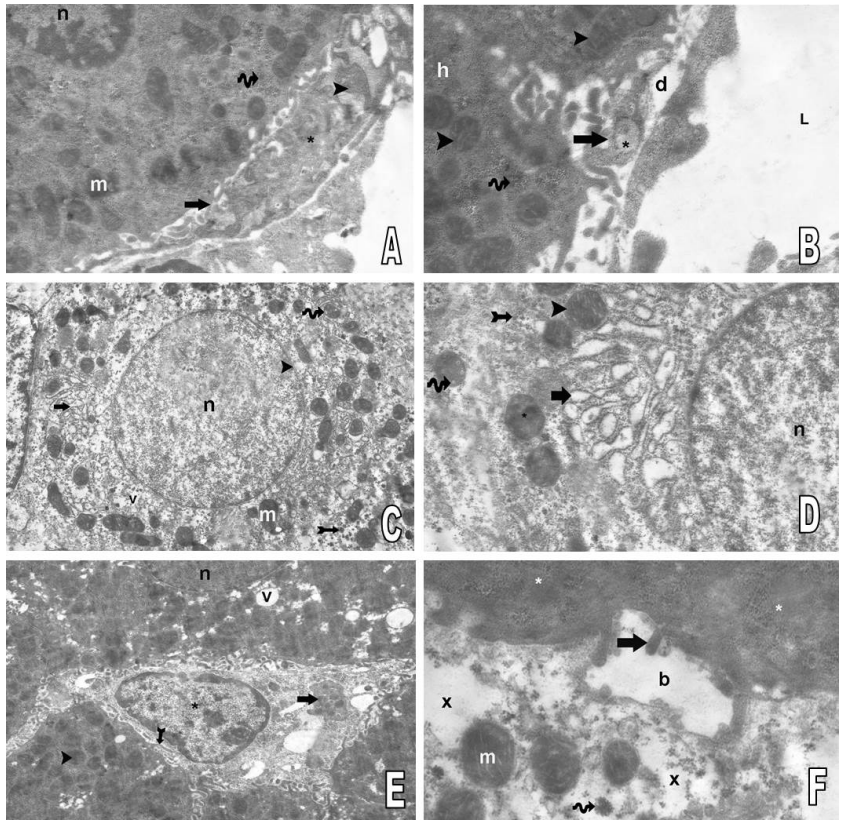

Figure 2. Transmission electron microscopic (TEM) photomicrographs of the liver in CGMR **(A** and **B)** and EGMR **(C–F)**. In CGMR, normal hepatocytes with regular nuclear structure (n in 2A) and cytoplasmic ultrastructure (h in 2B) and Disse space (d in 2B), clear mitochondrial cristae (m in 2A), alpha-glycogen granules (spiral arrow in 2A and 2B), stellate cell (arrow in 2A and 2B), process (arrow head in 2B) with lipid droplet (asterisk in 2B), and microvilli (arrow in 2A and 2B) can be seen. In EGMR, necrotic hepatocytes (x in 2F) with irregular nuclear structure (n in 2C) and dense cytoplasm (asterisk in 2F) with vacuoles (v in 2E) can be seen. Cytoplasmic (v in 2C) and mitochondrial (m in 2C and 2F; arrow head in 2D and 2E) vacuoles (spiral arrow in 2C and 2D), enlarged endoplasmic reticulum (arrow in 2C and 2D), alpha-glycogen (tail arrow in 2C and 2D; spiral arrow in 2F), stellate cell (asterisk in 2E) in Disse space, microvilli (tail arrow in 2E), part of fragmented hepatocyte (arrow in 2E), and bile canaliculi (b in 2F) with microvilli (arrow in 2F) were also seen in EGMR. CGMR, Control group male rats; EGMR, experimental group male rats (TEM: 10,000× for A; 15,000× for B and D; 6000× for C and E; 25,000× for F).

Table. Biochemical analysis results from liver tissues from CGMR and EGMR.

Biochemical parameters	CGMR (n = 6) (mean ± SD)	EGMR (n = 6) (mean ± SD)	P
MDA (nmol/mg tissue)	13.11 ± 0.83	18.31 ± 1.26 [a]	0.008*
SOD (mmol min^{-1} mg^{-1} tissue)	1.49 ± 0.05	1.77 ± 0.01[a]	0.008*
GSH (nmol/mg tissue)	1.20 ± 0.07	0.76 ± 0.02[b]	0.008*
CAT (mmol min^{-1} mg^{-1} tissue)	0.017 ± 0.001	0.018 ± 0.003[c]	1.00

*P < 0.05. Values with different superscripted letters are significantly different.
SD, Standard deviation; CGMR, control group male rats; EGMR, experimental group male rats; MDA, malondialdehyde; SOD, superoxide dismutase; GSH, glutathione; CAT, catalase

and immature rats exposed to long-term application of an 1800-MHz EMF (22). Şekeroğlu et al. (22) showed the irreversible effects of cytogenotoxic damage, especially in immature rats.

In growing rats, EMF at a frequency of 900 MHz has been reported to lead to moderate desquamation and vacuolization in the epithelium of testicular seminiferous tubules, while at a frequency of 1800 MHz it led to pathological findings such as severe vacuolization, necrosis, and desquamation in the seminiferous tubule epithelium (23). Odacı et al. (24) determined a decrease in granular cell numbers in the dentate gyrus of rat pups exposed to a 900-MHz EMF in the prenatal period. Another study investigating the effects of EMF in the prenatal period reported a decrease in testicular seminiferous tubule diameter (25).

All these studies clearly show that EMFs applied in the prenatal period have adverse effects on various organs (26,27). However, the effects on the liver of EMF applied in the prenatal period are still unknown, due to the lack of sufficient studies on the subject. Gokcimen et al. (28) reported in their study of young male rats that a magnetic field led to sinusoidal dilatation in the parenchyma and periportal area of liver tissue. Sinusoidal expansion and vacuoles surrounded by membrane in light microscopy and TEM findings were reported in rabbit liver sections exposed to a 650-MHz EMF for 12 months in another study. In the group exposed to EMF for 18 months in that study, irregularity was observed in sinusoidal lumen diameters, the cell cytoplasm was empty and replaced by granules, chromatin was less condensed, and the space between the interior and exterior nuclear membrane expanded (29). Another study referred to EMF altering mitochondrial structures, particularly in hepatocytes (30).

EMF applied to chicken embryos was reported to lead to cytoplasmic degenerations in liver cells (31). Similar to Lahijani et al.'s (31) findings, necrotic hepatocytes with an irregular nucleus and irregular cytoplasm structure were also observed in EGMR pups in our study. Histopathological findings such as expansions in endoplasmic reticulum, vacuoles in mitochondria, and active stellate cell fibrosis observed in EGMR sections in our study show the adverse effects on the liver in the postnatal period of 900-MHz EMF applied in the prenatal period. Our microscopic evaluations also confirm our TEM findings, because significant hydropic degeneration was determined in the pericentral area (zone 3) in slides from the EGMR group.

One study involving an adult male rat model established that a 128-mT static magnetic field increased MDA, an indicator of oxidative stress, in the testes, and that this stress increased levels of 8-oxo-dG, a determinant of DNA injury (32). MDA and SOD levels have been reported to increase and GSH to decrease in rats exposed to EMF at a frequency of 945 MHz and with a SAR value of 11.3 mW/kg (31). High-frequency 4.7-T EMF has been shown to lead to LPO in mice by increasing TBARS (30).

Studies have reported that exposure to EMF increases free radical production in rats and leads to DNA damage and oxidative stress in the liver (33,34). Koyu et al. (35) reported that the oxidative stress established in the liver by a 900-MHz EMF led to hepatic injury and lipid peroxidation in association with P-450-mediated organic hydroperoxide metabolism activation of hydroxyl radicals. Another study reported that EMF applied to the liver caused a rise in bilirubin, MDA, and SOD levels and suggested that this increase in SOD activity might be associated with a rise in anion structures (36). Güler et al. (37) reported that a 50-kHz electric field led to a rise in TBARS levels in plasma, liver, lung, and kidney tissues and that this increase showed tissue damage. They also reported that a rise in SOD levels was an indicator of ROS production against this damage and that this showed the physiopathological dimension of the exposure (37). GSH is an important ROS scavenger, represents the first line in antioxidant defense, and protects the cells against oxidative damage. However, elevated oxidative stress compromises the adaptive mechanism by suppressing GSH. Studies have reported that a rise in SOD is a response to that suppression (38). Another study stated that GSH protects hepatic cells against the chemical and enzymatic effects of oxidative damage (28). The biochemical findings from our study are similar to those from these studies. The rise in MDA levels in our study shows the presence of oxidative stress. On the basis of our biochemical data, we think that the 900-MHz EMF that we applied in the prenatal period led to a decrease in levels of GSH, a good scavenger of ROS in the enzymatic antioxidant defense system, and increased SOD levels as a response to that inhibition. We therefore report that EMF applied in the prenatal period causes oxidative damage and changes in the antioxidant defense system in the rat pup liver.

In conclusion, a 900-MHz EMF applied in the prenatal period led to oxidative stress in the rat pup liver and increased SOD levels as a response to oxidative stress, and hepatocytes may appear as significant pathological findings and this may affect the development of the liver.

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