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Research Article

Effects of Electromagnetic Field (1.8/0.9 GHz) Exposure on Spleen in Rats

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Abstract

Aim: To evaluate potential effects of whole-body 900 and 1800 MHz electromagnetic field (EMF) exposure on the rat spleen.
Material and Methods: The study was conducted on 9 Sprague–Dawley rats. Pregnant rats were assigned into 3 groups: 900 MHz EMF-exposure, 1800 MHz EMF-exposure and controls.
Results: Under light microscope, myeloid series cells, erythrocytes and megakaryocytes were observed in all groups. In the red pulp, dilated sinusoids were observed in both 900 and 1800 prenatal 24-hour groups with more prominent findings in the 1800 prenatal 24-hour group. Fused white pulps were apparent in 900 group while there was increase in the irregular white pulps (varying in size) with destruction in the1800 group. Biochemical evaluation showed that spleenmalondialdehyde level was higher while glutathione level waslower in the 900 MHz-exposure and 1800 MHz-exposuregroups compared to controls ($p<0.05$ for both).
Conclusion: Based on our results, it was concluded that EMF exposure at prenatal period led pathological changes in thespleenof pups. Again, it was revealed that it led oxidative stress through enhanced lipid oxidation and altered antioxidant defense systems. We also demonstrated that these effects were more evident as the level of EMF was increased from 900 MHz to 1800 MHz.

Keywords: Electromagnetic field, spleen, rat model, experimental animal models

INTRODUCTION

The electromagnetic (EM) wave is an area generated from a radiofrequency source which radiates in spacee. Electronic devices that we use frequenty and for a long period in our daily lives expose us to EMF. Today, the vast majority o mobile phones operates at 900 MHz and 1800 MHz in the GSM systems in Europe (1). The high- or low-power EM waves generated by electronic devices have deleterious effects on the human body. The high-frequency EM waves causemediated by heat while low-frequency EM waves createsharmful effects due to chemical changes in tissues (2). It is well-known that EMF exposure can produce biological and functional changes in cells through thermal and non-thermal effects on tissues (2-5). In the literature, it was shown thatEMF causes oxidative stress by increasing reactive oxygen species in tissues, causing DNA damage, protein folding defects and disruption Ca+2-dependent cell signaling due to increase in free radical and Ca+2 (2,6).

Immune system starts developing at the early embryogenesis and it proceeds with hematopoietic cell production, migration and differentiation at prenatal period. The early phases are sensitive to exogenous effects such as EMF. The spleen has a crucial role in the institution of immune responses against infection. Thus, exogenous factors which negatively affect thymus and spleen development can also influence on the immune system development. Although there is no definitive evidence that the electromagnetic field has harmful effects onin the researches about possible effects of EMF on the immune system, there are no comprehensive studies to rule out potential harm definitely (7). There is an increasing interest on potential deleterious effects of EMF on the immunee systems, which is promoted by reports on mobile phones used extensively and the carcinogenicity of EMF exposure (8).

The radiation-induced oxidative stress generates reactive oxygen species (ROS) which may result in injuries at

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tissue level (9). ROS plays a pivotal role in oxidative damage in lipids, DNA and proteins (10). They can trigger lipid peroxidation and cause alterations in reduced glutathione (GSH) and malondialdehyde (MDA) levels. MDA is produced during the premier chain reactions which promotepolyunsaturated fatty acids oxidation. Thus, it is a reliable marker of oxidative stress in tissues (11). On the other hand, GSH is a marker of antioxidant status (11).

The effects of EMF on several tissues were investigated; however, to best of our knowledge, there is no study on effects of EMF on the spleen, an important part of the immune system, in the literature. Thus, we aimed to evaluate the potential effects of whole-body 900- and 1800-MHz EMF on the spleenic tissues of rat in our study.

MATERIAL AND METHOD

Study Design

This study was conducted at Recep Tayyip Erdoğan University, Medicine School. The study was approved by Local Ethics Committee on Experimental Animals (approval: 2015/44-45). All procedures were performed in accordance to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

The study conducted on 9 female pregnant Sprague Dawley rats (weighing 280-300 grams) which were maintained on light-dark cycle of 12-hours at a steady environment of temperature (20-22°C) and humidity 50-55% relative humidity. The animals were fed ad libitum with free access to tap water. This experimental study was designed and conducted in accordance to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Pregnant rats were randomly assigned into 3 groups: sham-exposure (controls, n=3), 900 MHz EMF-exposure (n=3) and 1800 MHz EMF-exposure (n=3). No radiation was given to the control rats. Rats were subjected to continuous (24 hours) radiation for 20 days in 900 MHz EMF-exposure and 1800 MHz EMF-exposure groups.

Gestational Period

Female rats were housed in plastic cages (36 cm x 23 cm x 21 cm in size) as being 3 rats in each cage. In addition, a single male rat was put into all cages for one day in each cage for mating; the male rats were removed from cages thereafter. The vaginal secretions of the rats were collected by instilling 0.5 ml normal saline using a pipette. A vaginal smear was prepared from samples collected, which was examined under light microscope. The rat was considered to be conceived in the presence of sperm in microscopic examination (day 0 of gestation). The rats were regarded as be pregnant if vaginal membrane was observed during vaginal inspection and no microscopic evaluation was performed.

Following mating, radiation was applied to 6 pregnant rats (experimental group) using a digital signal generator. Remaining pregnant rats (control group) received no radiation.

Exposure System and SAR Calculation

In our study, rats in 900 MHz and 1800 MHz EMF-exposure groups were maintained separately in different experiment boxes. The control rats were isolated electromagnetically. In our study, 900 MHz EMF was generated from RF source identical to source used in the study by Koyu et al. (Everest GSM Simulator (Model: 900CW4, Türkiye). However, 1800 MHz EMF was generated by a distinct RF source (Everest GSM Simulator) (12). At exposure period, monopole antennas of exposure systems was mounted as close as possible (nearly 2 cm to the head) to the rat. In the experiment boxes (shielding effectiveness: appoximately100 dB at 1800 MHz), RF source radiation was measured by a spectrum analyzer (PROMAX, AE-566) using varying probes. In RF sources, there is a control switch in the front panel to adjust power output. In the exposure systems, the antenna power output values were maintained at a level mimicking effects of celullar and digital communication devices used in extensively in the society.

The newborn rats (n=8) without EMF were assigned into each group. The newborn rats were housed under above-mentioned conditions over two monts. On postnatal day 60, blood samples were drawn; in addition, liver was removed. Rats were sacrificied under anesthesia using intraperitoneal ketamine hydrochloride (50 mg/kg Ketalar®; Pfizer İlaçları Ltd. Şti., Istanbul, Türkiye) plus xylazine (10 mg/kg Rompun®; Bayer, USA). The spleens were also removed for histopathological examination.

Histopathological Analysis

Tissue samples were fixed in a 10% formaldehyde (Sigma – Aldrich Chemie, Steinheim, Germany) for 72 hours. Then, spleen tissue samples were gradually dehydrated using ethanol series of 50%, 70%, 80%, 90%, 96% and 100% (Merck GmbH, Darmstadt, Germany). The samples were placed intoxylol solution (Merck GmbH, Darmstadt, Germany) thereafter and subjected to mordanting process. Following mordanting process, the tisssue samples were embedded into paraffinblocks (Merck GmbH, Darmstadt, Germany). Then, sections (4-5 µm in thickness) were obtained and stained with Harris hematoxylin (Merck GmbH, Darmstadt, Germany) and eosin G (Merck GmbH, Darmstadt, Germany). The preparations were evaluated using a light microscope (Olympus CX51, Olympus Corporation, Tokyo, Japan) equipped with a digital camera (Olympus CX41, Olympus Corporation, Tokyo, Japan).

Biochemical Evaluation

The spleens removed were washed in cold phosphate buffered saline. In addition, liver samples obtained were weighed and underwent homogenization in phosphate buffered saline (pH 7.4) over 1 minute. Tissue weight: homogenization buffer ratio was 1:10. The homogenate obtained was centrifuged using following parameters: speed: 4500 rpm; duration: 20 min; temperature: 4°C.

MDA assays: The MDA level was quantified using the technique described by Draper and Hadley. Firstly, 10% trichloroacetic (0.5 mL) acid was added into liver tissue homogenate (0.1 mL), which then vortexed. After incubation at 94°C over 15 minutes, the mixture was cooled in cold water, and centrifuged using following parameters: speed: 3000 rpm; duration: 10 min. Then, 0.675% 2-thiobarbituric

acid (0.4 mL) was added to supernatatn (0.2 mL). After incubation at 94°C for 15 min, absorbance was measured at 532 nm by a spectrophotometer (13).

GSH assays: The GSH level was quantified by the Ellman method. The Na2HPO4 (0.5 mL) was added to liver tissue homogenate (0.125 mL); then, Ellman reagent was added to mixture. Absorbance was measured at 412 nm by a spectrophotometer (14).

Statistical Analysis

Statistical analyses were performed using the IBM SPSS version 21 (SPSS Inc., Chicago, IL, USA). Nonparametric data obtained as a result of semi-quantitative analyses were expressed as median, 25% and 75% interquartile ranges. After making the analyzes using non-parametric Kruskal Wallis test followed by a Tamhane T2 test using the differences between the groups, the numerical data of the groups were analyzed. All differences associated with a chance probability of 0.05 or less were considered statistically significant. Parametric data was calculated as mean standard deviation. Differences between the groups were tested using one-way analysis of variance (ANOVA) followed by a Tukey HSD test (p values<0.05 were regarded as significant).

RESULTS

Histopathological Analysis

Light microscopic evaluation of the spleen revealed myeloid series cells, erythrocytes and megakaryocytes in all groups. In the red pulp, it was seen that there wa dilated sinusoids in both 900 and 1800 prenatal 24-hour groups as being more severe in the 1800 prenatal 24-hour (Figure 1A, C). There was impairment on the central artery wall in both groups (Figure 1B, D). Fused white pulps were apparently seen in 900 prenatal 24-hour group and irregular white pulps (at varying siz) with destruction were increased in 1800 prenatal 24-hour group.

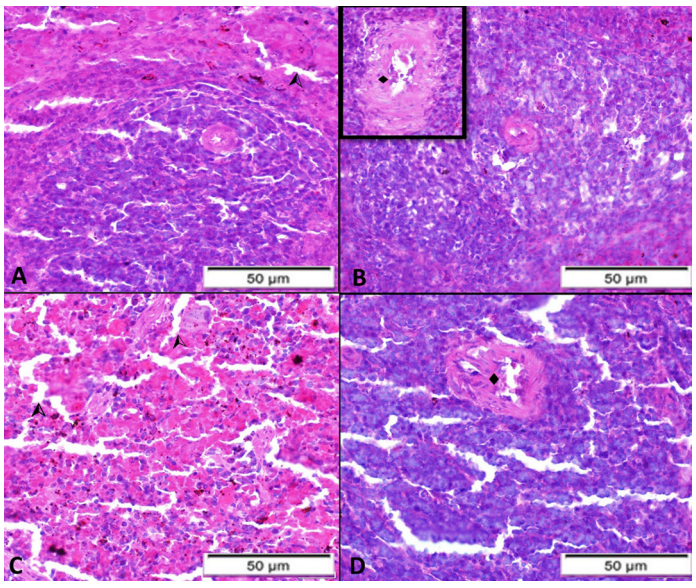

Figure 1. Light micrograph of the red and white pulps of the spleen sections in the 900 prenatal 24-hour group (1A, B), and in the 1800 prenatal 24 hour group (1C, D). Dilated sinusoids (×), are clearly seen. Inset (1B) demonstrate enlarged central artery with impaired arterial wall (®) (Hematoxylin and eosin, X50 µm)

Biochemical Results

In the biochemical evaluation, spleenic tissue MDA level wasfound to be higher in the 900 MHz EMF-exposure and 1800 MHz EMF-exposure groups than controls ($p<0.05$). Also, a significant difference was found in MDA levels in the 900MHz EMF-exposure group than those in 1800 MHz EMF-exposure group ($p<0.05$) (Table 1). The spleenic tissue GSH level was lower in the 900 MHz MF-exposure and 1800 MHz EMF-exposure groups than those in controls ($p<0.05$). Also, a significant difference was in GSH level in EMF900 group than EMF1800 group ($p<0.05$) (Table 1).

Table 1. Spleen tissue GSH and MDA levels in the control and EMF groups

	MDA (nmol/g protein)	GSH (nmol/g protein)
Control group	36.53 ± 0.68	8.60 ± 1.04
EMF900 group	41.04 ± 0.84	6.72 ± 0.43
EMF1800 group	48.54 ± 0.62	5.63 ± 0.64

EMF: electromagnetic field, MDA: malondialdehyde, GSH: glutathione
The results were given as mean±standard deviation

DISCUSSION

Although there are many studies about the prenatal effects of EMF on several tissues and organs of embryos and fetuses, our literature search revealed that there was no study on the splenic effects of EMF applied at 900-1800 MHz at prenatal period. Therefore, we aimed to evaluate whole-body 900- and 1800-MHz EMF exposure at prenatal period on the spleen inrats.

Specific absorption ratio-specific absorption rate (SAR) value has been determined and standards have been set in order to express the damages and heat effects of the radiation emitted by mobile phones and base stations in human tissues. Electromagnetic energy absorption, which increases the human body temperature by 1°C, is considered to be harmful to humans. This value is: 4 Watt/kg. One tenth of this value (0.4 W/kg) is considered as acceptable limit value for those exposed to electromagnetic fields because of their profession and 1/50 (0.08 W/kg) of this value is considered as acceptable limit value for general public exposure (15,16). In the literature studies, researchers report that biological functions change after 0.1 W/kg SAR value was delivered (17). It was reported that birth weights of pups were significantly decreased when pregnant Wistar albino rats were subjected to SAR value of 0.15 W/kg and emission of 890-915 MHz emission for 2 hours daily (18). In another developmental study, chicken embryos were exposed to mobile phone EMWs and mortality was calculated. It was reported that mortality rate was 70% in the EMW group vs. 16% in the controls (19). In our study, the rats were exposed to radio frequency up to 0.2 W/kg SAR value.

During prenatal period, exposure to environmental agents have significant effects on the thymus and spleen development and may threaten T cell-dependent responses in adults (20,21). Chagnaud and Veyret investigated

the effect of the GSM-tuned (900 MHz) microwave delivered 2 hours/day over tenconsecutive days, on the lymphocyte subgroups and normal mitogenic responses of Sprague-Dawley rats. Authors found no changes in the surface phenotypes or mitogenic activities of the spleen lymphocytes, suggesting no influence on immune system integrity (22). In the study on the effect of radiofrequency at GSM –tuned 900 MHz on mouse spleen cells, Gatta et al. found no change in total number of spleen cells, at B and T lymphocyte frequencies, and in CD69 and CD25 expression of T or B lymphocyte subgroups (23). Based on our histological results , dilated sinusoids in both 900 and 1800 prenatal 24-hour groups, being more severe in the 1800 prenatal 24-hour group were observed in the red pulp. Impairment on the central artery wall was evident in both groups. Fused white pulps were clearly observed in 900 group, and variable size of irregular white pulps with destruction were increased in 1800 group.

It is essential to keep oxidant-antioxidant systems in balance in the organism for maintaining health (24). Free radicals are endogeneous by products generated during the normal metabolic process. These radicals can interact with and cause injury in all types of cellular components (lipids, nucleic acidss and proteins in particular) as they reactive compounds. The increased free radical generation and/or failure of antioxidant defense system can result in xidative stress in living organism (25). MDA and GSH are well-known biomarkers for oxidative stress. MDA is produced during the premier chain reactions which promote polyunsaturated fatty acids oxidation. In their study, Esmekaya et al. reported elevation in MDA levels in heart, liver, testis and lung tissues resulting from 900 MHz EMF exposure (26). In the study by Aydin and Akar, effects of 900 MHz EMF on rat lymphoid organs, polymorphonuclear leukocytes and serum oxidative stress parameters were investigated. In agreement with our study, authors reported that MDA levels were significantly increased in spleen and thymus (27). Organisms have some antioxidant defense systems against oxidative injury resulting from formation of reactive oxygen species. By acting as a scavenger, GSH can remove ROS from organism and prevent oxidative damage resulting from ROS (28). In the present study, we found that spleen tissue MDA level was higher while GSH level was lower in EMF groups than in those in the controls. Also, there was significant differences were observed in MDA and GSH levels in the EMF900 group as compared with those in EMF1800 group.

There are major concerns about reliability of using animal models to investigate human toxicity; however, such concerns do not rely on the scientific grounds of translating results across species. Instead, they address soundpotentially correctable issues, such as technical competence in animal research, robustness of study design in animal experiments, and publication bias.

CONCLUSION

To best of our knowledge, this is the first study to investigate the effects of 900- and 1800-MHz EMF given at the prenatal period on the spleen. Based on the histological and biochemical results of the present study, it was concluded that EMF exposure at prenatal period led pathological changes in the spleenof pups. Again, it was revealed that it led oxidative stress through enhanced lipid oxidation and altered antioxidant defense systems. We also demonstrated that these effects become more evident as the level of EMF increases from 900 MHz to 1800-MHz.

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Conflict of Interest: *The authors declare that they have no competing interest.*

Ethical approval: *This study was conducted at* Recep Tayyip Erdoğan *University, Medicine School. The study was approved by Local Ethics Committee on Experimental Animals (approval#: 2015/44-45). All procedures were performed in accordance to National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.*

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