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The Effect of 900-Megahertz Electromagnetic Field Exposure in the First and Middle Adolescent Period on the Spleen in Male Rats: A Biochemical and Histopathological Study

İlk ve Orta Adolesan Dönemdeki Erkek Sıçanlara Uygulanan 900-Megahertz Elektromanyetik Alanın Dalak Üzerine Etkileri: Biyokimyasal ve Histopatolojik Çalışma

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ABSTRACT

Aim: Adolescents are at risk due to the intensive use of mobile phones. The aim of this study was to investigate the histopathological and biochemical effects of 900-Megahertz electromagnetic field on spleen in late adolescent period, exposed during periods of early and mid-adolescence.

Material and Methods: In this study, 24 Sprague Dawley 21-day-old male rats were divided into control (n=8), sham (n=8) and electromagnetic field groups (n=8). Control group rats were not subjected to any application. Electromagnetic field group rats were taken into the electromagnetic field cage and were exposed to 900-Megahertz electromagnetic field (1 hour per day for 25 days). Sham group rats were taken into the electromagnetic field cages but were not exposed to electromagnetic field. At the end of the treatment, all animals were sacrificed by cervical dislocation method and the spleens were removed. After histological procedures, tissue sections were taken and stained with hematoxylin-eosin and periodic acid Schiff. Histopathological evaluation was performed on the spleen tissues. Oxidative stress parameters including lipid peroxidation, superoxide dismutase, glutathione and catalase levels were investigated via biochemical analysis.

Results: Histopathological evaluation revealed megakaryocyte cells, enlarged white pulps and dilated sinusoids in spleen tissues of adolescent rats in electromagnetic field group. According to biochemical analysis results, it was determined that glutathione and lipid peroxidation values were increased, but superoxide dismutase and catalase values were decreased.

Conclusion: It can be said that the 900-Megahertz electromagnetic field applied in adolescent period caused morphological changes on spleen tissue and caused oxidative stress in male rats.

Keywords: Adolescent; spleen; electromagnetic field; oxidative stress.

ÖZ

Amaç: Adolesanlar cep telefonlarının yoğun kullanımından dolayı risk altındadır. Bu çalışmanın amacı ilk ve orta adolesan dönemlerde maruz kalınan 900-Megahertz elektromanyetik alanın, geç adolesan dönemde dalak üzerindeki histopatolojik ve biyokimyasal etkilerini araştırmaktır.

Gereç ve Yöntemler: Bu çalışmada 24 adet 21-günlük Sprague Dawley tipi adolesan erkek sıçan, kontrol (n=8), sham (n=8) ve elektromanyetik alan (n=8) gruplarına ayrıldı. Kontrol grubu sıçanlara herhangi bir uygulama yapılmadı. Elektromanyetik alan grubuna ayrılan sıçanlar, elektromanyetik alan kafesi içerisine alındı ve 900-Megahertz elektromanyetik alana (25 gün boyunca her gün 1 saat) maruz bırakıldı. Sham grubu sıçanlar ise elektromanyetik alan kafesine alındı fakat elektromanyetik alana maruz bırakılmadı. Uygulamaların bitiminde tüm hayvanlar servikal dislokasyon yöntemiyle sakrifiye edilerek dalakları çıkarıldı. Histolojik işlemlerden sonra, dokulardan kesitler alındı ve hematoksilen-eozin ve periyodik asit Schiff tekniğiyle ile boyandı. Dalak dokularında histopatolojik değerlendirme yapıldı. Biyokimyasal analizler ile oksidatif stres parametrelerinden lipit peroksidasyonu, süperoksit dismutaz, glutatyon ve katalaz düzeyleri incelendi.

Bulgular: Histopatolojik değerlendirmede elektromanyetik alan grubu adolesan sıçanların dalak dokularında megakaryosit hücreler, genişlemiş beyaz pulpalar ve dilate sinüzoidaller izlendi. Biyokimyasal analiz sonuçlarına göre glutatyon ve lipit peroksidasyonu değerlerinin arttığı, ancak süperoksit dismutaz ve katalaz değerlerinin azaldığı tespit edildi.

Sonuç: Adolesan dönemde uygulanan 900-Megahertz elektromanyetik alanın erkek sıçanların dalak dokusu üzerinde morfolojik yapıda değişiklikler meydana getirdiği ve oksidatif strese neden olduğu söylenebilir.

Anahtar kelimeler: Adolesan; dalak; elektromanyetik alan; oksidatif stres.

INTRODUCTION

As the use of apparatus emitting electromagnetic field (EMF) increases (cellular phones, base stations, broadcast antennas, Wireless Fidelity (WiFi), etc.), environmental exposure to the EMF increases. Mobile phones have been the focus of researchers because of their widespread use as well as their close proximity during their use to the human body. The overuse of mobile phones by the adolescents is predicted to result in an increased EMF influence on their tissues, which are still developing. The spleen is a lymphoid organ that acts as a biological sieve where macrophages mature and interact with T and B cells (1). Any exposure that would disturb the development of the spleen will affect the development of the whole immune system. This risk will increase further, especially considering that the development of tissues continues during adolescence. There are many factors that cause oxidative stress in the spleen, one of which is EMF (2,3). Cells have different mechanisms to overcome oxidative stress and repair damaged macromolecules. The primary defense mechanism is mediated by the antioxidants that have been shown to remove free radicals and reactive oxygen species (ROS), enzymatically or non-enzymatically. It has been previously shown that antioxidant enzymes like catalase (CAT) and superoxide dismutase (SOD), as well as non-enzymatic antioxidants such as glutathione (GSH) are significantly affected under oxidative stress (1).

A comprehensive analysis of the previous studies suggest that exposure to EMF could cause significant physiological, pathological changes and behavioral disorders in children. It is highly likely that adolescent children, whose tissues and organs are yet to be completely developed, will be impacted by EMF and exposed to its pathological effects more than adults. These notions make us wonder the effects of EMF exposure in adolescence. Tirelli et al. (4) describes adolescence as the period of 21-59 days in rodents. They have categorized the adolescent period into three age-intervals as early (21-34 days), mid (34-46 days), and late (46-59 days) adolescence.

There is no consensus within the scientific community over the effects of exposure to EMF. Some researchers propose negative effects of EMF (5-10), while some others claim that EMF has no effect (11,12) or even is beneficial (14-16). In light of all these studies, here in this paper we investigated the histological and biochemical effects of 900 Megahertz (MHz) EMF exposure to male rats during early and middle adolescence, on the spleen tissue in late adolescence.

MATERIAL AND METHODS

Laboratory Settings, Groups and Ethics Statement

All animal procedures were approved by the Karadeniz Technical University Animal Experiments Local Ethical Committee (Date: 19.06.2014, Protocol Number: 2014/30) and were carried out according to the principles of the Guide for the Care and Use of Laboratory Animals. The animals were housed in Karadeniz Technical University experimental animals laboratory. The animals were kept in an automatically adjusted 12 hour light and 12 hour dark cycle, with an average temperature of 22±2 ºC and 50±5% humidity. Tap water and standard rat chow (Bayramoglu Feed Industry and Trade CO., Erzurum, Turkey) were used. In this study, a total of 24 Sprague Dawley 21-day-old male rats of were divided into three groups equally with 8 rats in each group as control group (C-G), sham group (S-G) and electromagnetic field group (EMF-G). No treatment was applied to the C-G rats. EMF-G rats were taken into the EMF cage every day at the same time (between 10.00-11.00 am) and were exposed to EMF of 900 MHz (1 hour per day for 25 days). S-G rats were taken into the EMF cages in the same schedule throughout the experiment but not exposed to EMF.

Electromagnetic Field Application System

This system has previously been used in many studies (2,6,8,12,13,17-19). Cage used for EMF application was made of plexiglas material. The cage dimension was 30x42x50 cm. Also, the cage had a 126 cm base area. A high-speed oscillator with an output power of approximately 300 mW and a frequency set to 900 MHz was inserted into the cage for the generation of 900 MHz EMF (1218-BV, Lockable Oscillator, 900-2000 MHz, General Radio Company, Concord, Massachusetts, USA, Serial No. 1483). A stationary uninterrupted power supply was used for both the operation of the oscillator and the continuous supply of energy (1267-B Regulated Power Supply, General Radio Company, Concord Massachusetts, USA, Serial No. 903). The output of the oscillator was connected by a coaxial cable to a half-wave 15 cm-long and 1 mm-wide copper dipole antenna. The antenna was placed in the middle, approximately 11 cm inside from the top open surface. During the EMF application, the electric field intensity was measured at different points of the cage using a broadband field intensity-meter with a measuring range of 100 Kilohertz (kHz)-2.5 GHz (C.A 43 Isotropic Electrical Field Intensity Meter, Chauvin Arnoux Group, Paris, France). Intermediate values outside the measurement points were determined by interpolation (SAR: specific absorption ratio, Rad Haz SAR Equivalency Calculator Version 1.0, Richard Tell Associates, Inc., Mesquite, NV). The mean electric field intensity, the power intensity and the SAR value were calculated as 8.8 V/m, 0.21 W/m^2 and 0.0395 W/Kg, respectively.

Histological Analyses

Spleen tissues were formalin-fixed and paraffin embedded through histological procedures. 5-micrometer sections in thickness were cut using microtome (Thermo Scientific Shandon Finesse 325 microtome, UK). Spleen tissues were stained with hematoxylin-eosin (H&E) and periodic acid Schiff (PAS), then evaluated at x60 magnification under the light microscope. BX53 light microscope was used for histopathological examinations and a DP 72 camera was used for microscopy (Olympus Optical Co., Tokyo, Japan).

Biochemical Analyses

Tissue samples (0.5 g) were taken from each animal and homogenized in 4.5 ml of suitable buffer. SOD, CAT, GSH and lipid peroxidation (LPO) levels were assessed in the spleen tissues. LPO determination was performed using thiobarbituric acid test and the results were reported as nmol MDA/mg tissue (20). The presence of CAT was measured via H_2O_2 dissociation at 240 nm and the results were reported as µmol/min/mg tissue (21). SOD was measured in accordance with the method developed by Sun et al. (22) and the results were given in mmol/min/mg

tissue. The GSH amount in the tissues was determined via the method of Sedlak and Lindsay (23) with modifications and the results were given in nmol/mg tissue units.

Statistical Analysis

Distribution of variables were checked by Shapiro-Wilk test and histogram graph. One way ANOVA test was used for group comparisons since the normality and variance homogeneity assumptions were met. Then, Tukey post hoc test was applied for pairwise comparisons. Descriptives were calculated as mean±standard deviation. SPSS v.22 statistical package was utilized for statistical analysis and $p<0.05$ was considered statistically significant.

RESULTS

Histopathological Evaluation of the Spleen Tissue

Sections taken from spleen tissues of all groups were stained with H&E and PAS, and were evaluated histologically under the light microscope. Sections taken from the EMF-G were compared with the sections from the C-G and S-G. H&E staining of the spleen sections showed megakaryocytes, enlarged and fused white pulp and enlarged sinusoid in the EMF-G rats. No pathology was observed in the spleen sections of C-G and S-G (Figure 1 and 2). Megakaryocytes, erythrocytes and myeloid series cells were observed in the PAS-stained sections of the EMF-G. No pathology was found in the spleen sections of C-G and S-G (Figure 3).

Findings on the Biochemical Parameters

There were significant differences between groups for all biochemical parameters, LPO ($p=0.001$), GSH ($p<0.001$), CAT ($p=0.027$) and SOD ($p<0.001$). According to post hoc test results, significant increases were found in terms of LPO ($p=0.001$) and GSH ($p=0.022$) activities while CAT ($p<0.001$) and SOD ($p<0.001$) activities were found significantly lower in the EMF-G when compared to C-G. In addition, SOD ($p<0.001$) activity was found statistically lower in the S-G compared to C-G, while there were no significant differences between S-G and C-G in terms of LPO ($p=0.827$), GSH ($p=0.461$) and CAT ($p=0.192$) activities. When EMF-G compared with S-G, a significant increases was found in LPO ($p=0.004$) activity, but the increase in GSH activity was not found statistically significant ($p=0.199$). And both the decreases in CAT ($p<0.001$) and SOD ($p=0.027$) activities were found significant in EMF-G compared to S-G (Table 1).

DISCUSSION

Devices that emit EMF have become an integral part of our life, which has brought about the desire to investigate the health-related effects of EMF as well. There are several studies in the literature that are associated with negative effects of EMF exposure on health (5-9,16-18,24-28). However, there are only a limited number of studies that delve into the effect of EMF on the spleen.

Tissues are protected themselves against oxidative stress-inducing damages via enzymatic antioxidant defense mechanisms such as CAT and SOD as well as antioxidant defense mechanisms, which are non-enzymatic like GSH. Any circumstance that will disrupt this harmony will result in oxidative stress in the tissues. One such reason that disrupts this harmony and causes oxidative stress in tissues is exposure to EMF. In line with this, previous studies have shown that EMF application for different durations and with

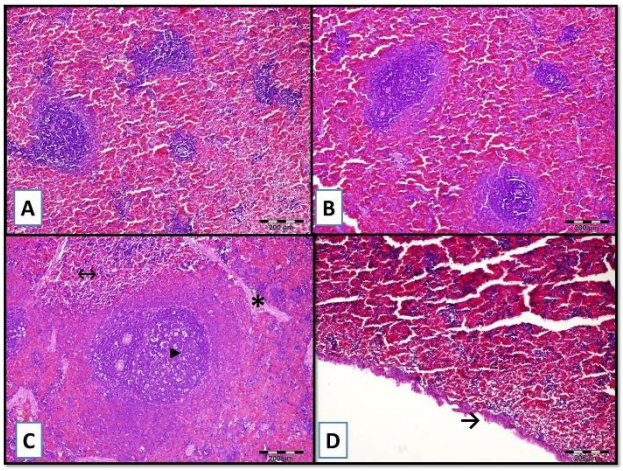

Figure 1. Microscopic view of the spleen in control (A), sham (B) and electromagnetic field (C,D) group rats (x20). (→) Capsule, (*) Trabeculae, (↔) White pulp and (►) Red pulp. Spleen sections were stained with hematoxylin-eosin. The spleen appears normal in A and B sections. C section shows enlarged and fused white pulp. D section displays normal spleen membrane structure.

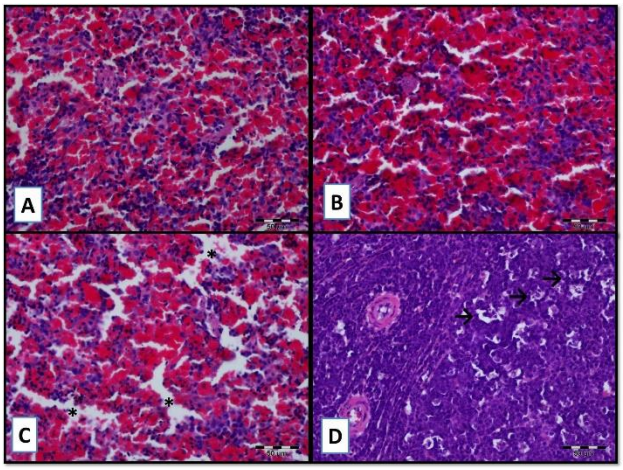

Figure 2. Microscopic view of the spleen in control (A), sham (B) and electromagnetic field (C,D) group rats (x60). Spleen sections were stained with hematoxylin-eosin. C section indicates (*) dilated sinusoids and D section shows (→) megakaryocyte cells.

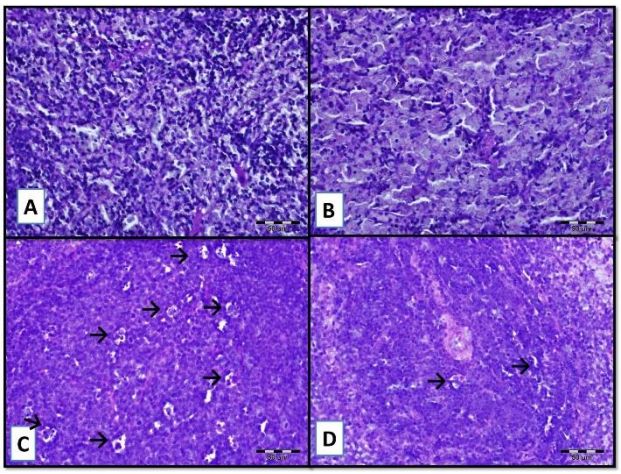

Figure 3. Microscopic view of the spleen in control (A), sham (B) and electromagnetic field (C,D) group rats (x60). Spleen sections were stained with periodic acid Schiff. The spleen appears normal in A and B sections. C and D sections show (→) megakaryocytes, erythrocytes and myeloid series cells.

Table 1. Biochemical parameters of spleen tissues

Biochemical Parameters	C-G (n=8)	S-G (n=8)	EMF-G (n=8)	p
Lipid peroxidation (nmol/mg tissue)	35.26±1.57[a]	38.55±7.33[a]	59.89±14.92[b]	**0.001**
Catalase (µmol/min/mg tissue)	0.34±0.04[a]	0.37±0.03[a]	0.11±0.01[b]	**<0.001**
Superoxide dismutase (mmol/min/mg tissue)	8.84±0.32[a]	6.40±0.26[b]	5.70±0.59[c]	**0.027**
Glutathione (nmol/mg tissue)	4.40±0.29[a]	4.64±0.23[ab]	4.99±0.45[b]	**<0.001**

C-G: Control group, S-G: Sham group, EMF-G: Electromagnetic field group, a,b,c: Different superscript letters denote significant differences between the groups.

different intensities cause oxidative stress in the spleen tissue (2,3,27). EMF causes depletion in the amount of antioxidants in the spleen, which consequently results in oxidative stress and suppression of hepatic and immune function in the spleen (3). In this study, we observed that the levels of LPO in the spleen tissues of EMF-G rats were increased. This increased level indicates that EMF exposure causes oxidative stress in the spleen. In addition, CAT and SOD enzyme activities were significantly reduced but there was an increase in the GSH levels of the EMF-G rats. GSH levels are normally expected decreased; however, there are studies suggesting increased GSH levels in tissues after prolonged exposure to EMF (29,30). Similar to our findings, Li et al. (3) showed oxidative stress induction due to EMF application with different intensities, accompanied by decreased SOD and CAT values. Furthermore, GSH level was increased in the long-term pulsed EMF while it was decreased in control group. The microscopic evaluations in this study show that the 900 MHz EMF applied to male rats in adolescent period caused alterations in the spleen tissue. Images obtained from the EMF-G spleen tissue sections indicated enlarged sinusoids, enlarged and fused white pulp, megakaryocytes, erythrocytes and myeloid series cells. Similar studies such as Kamel et al. (31) reported the multi-nucleated giant cell types in the spleen tissue as megakaryocytes (polykaryocytes). In another study, it was shown that EMF applied at different intensities caused degeneration of the spleen tissue and loss of megakaryocytes and monocytes (28). Moreover, another study evaluating 21 day postpartum spleen tissue after EMF application during the prenatal period revealed the presence of cells with large eosinophilic, granular cytoplasm and cells that are similar to oncocytic cells with round and oval nuclei, as well as megakaryocytes, erythrocytes and myeloid series cells (2). A study evaluating the effects of EMF with different intensities on spleen tissue under the light microscope detected dilatation in the sinusoid cavities and the white pulp, together with disruptions in the white pulp appearance; which were explained by hyperplasia of the lymphoid tissue (32). On the other hand, in a study conducted by a group of researchers, it was stated that EMF had no negative biochemical and histological effects on the body (11).

In conclusion, we determined that 900MHz EMF applied to adolescent male rats caused pathological changes in their spleen tissue, accompanied by increased levels of LPO, which affected the antioxidant defense systems and caused oxidative stress.

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