

Effects of 50 Hz electric field on malondyaldehyde and nitric oxide levels in spinal cord of rats at prenatal plus postnatal period

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Aim: The effect of 50 Hz electric field (EF) on developing neural tissues is not known. We aimed to determine whether there would be any oxidative stress effect of 50 Hz EF on developing spinal cord tissues of pup rats by evaluating malondialdehyde (MDA), nitric oxide (NO) levels, and antioxidant enzyme activities such as superoxide dismutase (SOD) and catalase (CAT) measurement.

Materials and methods: In the prenatal+postnatal group, the pregnant rat was exposed to continuous EF at 50 Hz 10 kV/s until delivery. Then the female pups of this group (n = 7) were exposed to EF at the same doses and for the same duration until puberty. In the postnatal group, the pregnant rat was unexposed to EF during pregnancy, but the female pups from this group (n = 7) began to be exposed to EF at delivery, and continued to be exposed to EF at 50 Hz EF 10 kV/m for 24 h until puberty.

Results: In the prenatal+postnatal group, the MDA and NO levels in the spinal cord tissue of pup rats were significantly higher when compared to the control group. However, in the postnatal group, the MDA and NO levels in the spinal cord tissue of pup rats were not significantly different when compared to the control group.

Conclusion: A 50 Hz electric field may cause oxidative stress in the spinal cord of rats in the prenatal+postnatal period.

Key words: Electric field, oxidative stress, nitric oxide, malondialdehyde, spinal cord

Doğum öncesi ve doğum sonrası dönemde 50 Hz elektrik alanının rat spinal kord malondialdehid ve nitrik oksit seviyelerine etkisi

Amaç: Gelişmekte olan nöral dokular üzerine 50 Hz elektrik alanının etkisi bilinmemektedir. SOD ve katalaz gibi antioksidan enzim aktiviteleri, malondialdehid ve nitrik oksit seviyelerini değerlendirerek yavru ratların gelişmekte olan spinal kord dokuları üzerinde 50 Hz elektrik alanının oksidatif stress etkisinin olup olmadığını amaçladık.

Yöntem ve gereç: Prenatal + postnatal grupta gebe ratlar doğuma kadar günde 24 saat sürekli olarak 50 Hz 10 kV/s elektrik alana maruz kaldı. Doğumdan sonra bu grubun dişi yavruları aynı doz ve sürede elektrik alana maruz bırakıldı (n = 7). Postnatal grupta gebe rat gebelik sırasında elektrik alana maruz kalmadı. Fakat bu grubun dişi yavruları doğumda elektrik alana maruz kalmaya başladı ve yavru dişi ratlar puberteye kadar elektrik alanına sürekli olarak maruziyete devam edildi (n = 7).

Bulgular: Yavru dişi ratların spinal kord dokularında MDA ve NO seviyeleri prenatal + postnatal grupta kontrol grubuna göre anlamlı derecede yüksekti (P < 0.05). Fakat postnatal grup ile kontrol grubu karşılaştırıldığında spinal kord dokularında MDA ve NO seviyelerinde anlamlı fark saptanmadı (P > 0.05).

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Sonuç: Prenatal ve postnatal dönemde 50 Hz elektrik alanına maruziyet ratların spinal kordunda oksidatif strese neden olabilir.

Anahtar sözcükler: Elektrik alan, oksidatif stres, nitrik oksit, malondialdehid, spinal kord

Introduction

The biological effects of an extremely low frequency electric and magnetic field have become a matter of interest (1). Due to the advent of novel technology in medical diagnostic and therapeutic tools, electromagnetic pollution has occurred due to static and extremely low-frequency (ELF) and electromagnetic fields (EMF) (2). Both electric and magnetic fields and their related currents may have an important adverse effect on various biological functions (3-5). Many experimental studies have been performed on the effects of electric and magnetic fields on our health (2,6). However, because of the environmental conditions, in real life we are sometimes exposed to pure EF without magnetic field (7). For example, people are exposed to EFs generated by high voltage power lines with no current, mine processing plants using static electric as a power source, or idling high voltage power transformers (7,8).

Many effects on biological systems exposed to static and ELF fields have been investigated (2). Guler et al. (1) found that thiobarbituric acid reactive substances levels, and SOD activities of serum increased in the guinea pigs exposed to 50 Hz EF. Okudan et al. (9) reported that the static and 50 Hz EF influences the development of rat bones especially when they are exposed to EF during their intrauterine and neonatal period, by using DEXA analysis. Aydın et al. (10) suggested that that continuous 50 Hz EF exposure has a weak effect that is detrimental mostly to the rate of early nerve regeneration in a crush injury model. It has been reported that electric field induced lipid peroxidation (LPO) by increased malondialdehyde (MDA) in various tissues such as brain, plasma, lung, and hepatic tissue (3,6). MDA is a breakdown product of some major chain reactions leading to oxidation of polyunsaturated fatty acids and thereby it serves as a reliable marker of oxidative stress-mediated LPO (11,12). However, to our knowledge, no study has been performed so far

considering the oxidative stress effects of EF on the spinal cord of rat pups.

EFs in our daily life cause an increase in ROS levels in tissues, resulting in oxidative stress (1). However, the effect of 50 Hz EF on developing neural tissues is not known. We studied whether EF would have any oxidative stress effects on developing spinal cord tissues of rat pups by evaluating MDA, NO levels, and antioxidant enzyme activities such as SOD and CAT measurement.

Materials and methods

Study Design

The study was approved by the Institutional Review for Animal Research Board of Süleyman Demirel University and conducted in accordance with the institutional guidelines. Five-month-old female Wistar albino rats were mated with male rats in the study. Each rat was placed into a cage with a male rat overnight and the first 24 h period following the mating procedure was designated as day 0 of pregnancy. The pregnant rats and their pups were randomly assigned to 3 groups. Firstly, all pregnant rats were placed into 1 cage and they were randomly distributed among 3 groups as the prenatal+postnatal, the postnatal and the sham-exposed (control) groups. The pregnant rat of the prenatal+postnatal group was continuously exposed to EF at 50 Hz EF 10 kV/m for 24 h during pregnancy. Their female pups continued to be exposed to EF after delivery. The pups from the prenatal+postnatal (n = 7) continued to be exposed to EF at 50 Hz EF 10 kV/m for 24 h until puberty. In the postnatal group, the pregnant rat was unexposed to EF during pregnancy, but the female pups from this group (n = 7) began to be exposed to EF at delivery, and continued to be exposed to EF at 50 Hz EF 10 kV/m for 24 h until puberty. The 3rd group was the sham-exposed group. In this group, the pregnant rats were sham exposed to EF during pregnancy. After delivery, the female pups selected from the control group (n = 6) were sham exposed to EF until puberty.

The weight of the pups was measured weekly and genital examination was performed twice a day to determine vaginal opening after the first 15 days of their birth. Vaginal smear was taken to evaluate cornified epithelium after vaginal opening. The day of vaginal opening and determination of cornified epithelium were recorded as the time of onset of puberty. The blood samples were obtained and the rats were decapitated when vaginal opening and estrous were detected.

All pups were fed with breast milk and standard food during the first 21 days of their life. After weaning, the rats in all groups were continued to be fed with standard food. All animals were subjected to normal daylight cycles (0800-2000) at room temperature (21-22 °C). During the experiments, all animals were provided the same feeding and watering process. The groups were kept at the same environmental conditions [humidity (55%-60%), temperature, light intensity, and electromagnetic fields]. In the same room, the sham-exposed, the prenatal+postnatal, and the postnatal group were separated by custom-made stainless steel shield (Figures 1 and 2). According to the standards and regulations of Institute of Electrical and Electronic Engineers and World Health Organization power frequency exposure limit is 10 kV/m (13,14). For this reason, we decided to use of about 10 kV/m EF intensity in power frequency, 50 Hz pure EF condition in this study. All animals were exposed to EF for 24 h. Namely, these animals were exposed to EF during their whole life in the experimental room.

At the end of the study, all pups were decapitated under ether anesthesia. The spinal cords were quickly removed from the thoracic and lumbar area. The

spinal cord tissues of the pups were stored at -20 °C until biochemical analysis.

Electric Field Application Setup

Exposure system

A custom-made parallel plate exposure system was utilized. It was planned, constructed, and tested in the Antennas and Propagation Laboratory of Department of Electronics and Communication Engineering at Süleyman Demirel University. From the basic electromagnetic theory, the field lines in a set of parallel-plate and the field between them is highly uniform. The plates' effective area is 0.5 m² (0.5 × 1.0 m) and the space between the plates is (variable 0.1-1.0 m) to avoid changing value of field between the plates at power frequency range (14). This setup is suitable to be used for small animal studies because the cage's size for 6-8 animals is about 40 × 50 × 20 (w × l × h) in cm with 2 mm thickness. A perfectly conductor (stainless steel) was used in the construction of the plates because free surface charges can be homogeneous to get uniform fields. The specific rate (SAR) values are known to have originated from absorbed RF (radio frequency) energy in tissues. In power frequencies (50 Hz/60 Hz) thermal effects are not the dominant factor (14). Because of this, SAR values of animals were not calculated.

The electric field application setup is shown in Figures 1 and 2. The EF strength was calculated according to the equation $E = V/d$, where E is the electric potential between the plates, d is the distance, and E is the EF intensity in V/m. The corners were rounded to avoid corruption due to the end effect. They were placed upright on wooden stands and positioned parallel to each other. In order not to

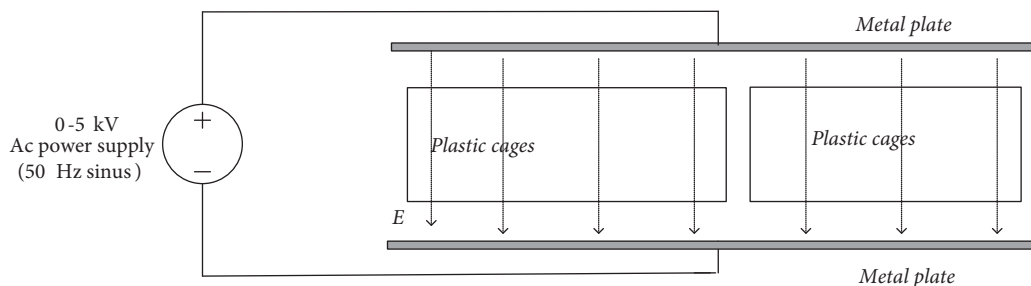


Figure 1. The schematic view of the electric field application setup.

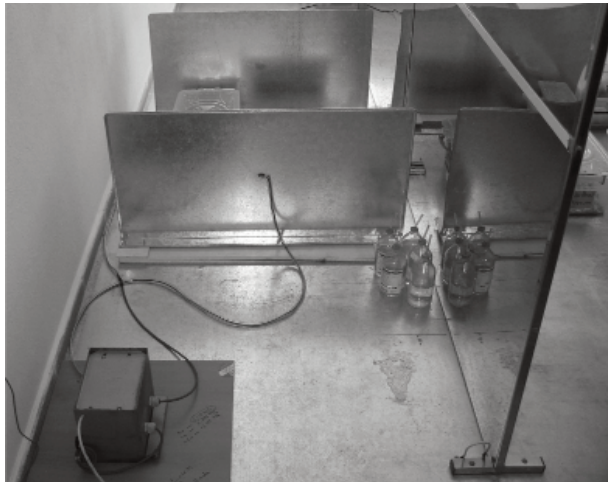


Figure 2. The appearance of the electric field application setup.

disturb the field, the leads were connected to the center of the plates on their outer sides (13,14).

In the exposure system, a step-up transformer rated 220 Vrms/5000 Vrms and 1000 VA was used. The plates were spaced at 50 cm apart. The animals were free in their well-matched plastic cages. In addition to weekly cleanups of the plates and cages, the setups were frequently scrutinized for gross soil and wetness that would disturb the homogeneity of EF. The attenuation due to routine soiling could be estimated to cause very small variation in EF. From the equation above, average EF density between the plates was calculated to be $5000 \text{ V}/0.5 \text{ m}=10000 \text{ V/m}$ (10 kV/m) in the EF group. A multimeter (Max 3000 TRMS Model Chauvin Arnoux, Paris, France) was used for voltage measurements. The primary voltage of power transformer, the secondary voltage, and the EF density were in the range of 219-229 Vrms, 4975-5202 Vrms, and 9951-10405 V/m, respectively. A Digital Gauss/Tesla Meter (Unilab, Blackburn, England) was utilized to test the purity of EF from background magnetic fields. The maximum magnetic field density was about 0.001 mT. In the experiment room, unwanted high-frequency fields were tested by using an HI-3804 Electromagnetic Field Survey Meter-Industrial Compliance Meter and its probe (Holaday Industries, Inc., Eden Prairie, MN, USA).

Biochemical Analysis

The frozen tissue samples of spinal cord were thawed, weighed, and homogenized (Ultra Turrax

T25, Germany) (1:10, w/v) in 50 mM/L phosphate buffer (pH 7.4), keeping them in an ice bath. The homogenate was then centrifuged at $5000 \times g$ for 30 min to obtain supernatants.

The tissue protein was determined using the Lowry method (15). The MDA levels, an indicator of free radical generation increasing at the end of the lipid peroxidation, were estimated by the double heating method of Draper and Hadley (16). The principle of this method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. For this purpose, 2.5 mL of 100 g/L trichloroacetic acid solution was added to 0.5 mL supernatant in each centrifuge tube and the tubes were placed in a boiling water bath for 15 min. After cooling them in tap water, the tubes were centrifuged at $1000 \times g$ for 10 min and 2 mL of the supernatant was added to 1 mL of 6.7 g/L. The TBA solution in a test tube was placed in a boiling water bath for 15 min. The solution was then cooled in tap water and its absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Japan) at 532 nm. The MDA was expressed as nanomoles per gram wet tissue.

The total (Cu-Zn and Mn) SOD activity was determined according to the method of Sun et al (17). The principle of this method is based, briefly, on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide generator. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. The SOD activity was expressed as units per gram protein.

The CAT activity was measured according to the method of Aebi (18). The principle of the assay is based on the determination of the rate constant k (dimension: s^{-1} , k) of hydrogen peroxide decomposition. By measuring the absorbance change per minute, the rate constant of the enzyme was determined.

The NO measurement is very difficult in biological specimens, because it is easily oxidized to nitrite (NO^-) and subsequently to nitrate (NO^{3-}), which serve as index parameters of NO production. The method for spinal cord nitrite and nitrate levels was based on the Griess reaction (19). After samples were deproteinized

with Somogyi reagent, total nitrite (NO^{2-} NO^{3-}) was measured by spectrophotometer (Shimadzu, UV-1601, Japan) at 545 nm after conversion of NO^{2-} to NO^{3-} by copperized cadmium (Cd) granules. The results were expressed as micromole per gram wet tissue ($\mu\text{mol/g}$ wet tissue).

Statistical analysis

The data were presented as means \pm standard deviation (SD). SPSS 9.0 was used for the statistical analysis. One-way analysis of variance (ANOVA) and post-hoc multiple comparison tests (LSD) were performed on the data of biochemical variables to examine the differences among the groups. $P < 0.05$ was considered statistically significant.

Results

The results are shown in Table. In the prenatal+postnatal group, the MDA levels in spinal cord tissue were significantly higher when compared to the control group (13.941 ± 5.675 and 7.288 ± 2.917 nmol/g wet tissue, respectively) ($P = 0.009$). In the postnatal group, the MDA levels in spinal cord tissue were not statistically significant when compared to the control group (8.389 ± 2.553 and 7.288 ± 2.917 , respectively) ($P = 0.629$). The MDA levels of the prenatal+postnatal group were significantly higher when compared to the postnatal group (13.941 ± 5.675 and 8.389 ± 2.553 , respectively) ($P = 0.019$). In the prenatal+postnatal group, the NO levels in spinal cord tissue were significantly higher when compared

to the control group (5.498 ± 1.379 and 3.578 ± 1.718 , respectively) ($P = 0.02$). In the postnatal group, the NO levels in spinal cord tissue were not statistically significant when compared to the control group (3.637 ± 0.891 and 3.578 ± 1.718) ($P = 0.93$). The NO levels of the prenatal+postnatal group were significantly higher when compared to the postnatal group in the spinal cord of rats (5.498 ± 1.379 and 3.637 ± 0.891 , respectively) ($P = 0.019$).

The SOD and CAT activities were lower in the prenatal+postnatal group when compared to the control group, but they were not statistically significant (the SOD activities 0.053 ± 0.01 vs. 0.071 ± 0.02 U/g protein and the CAT activities 0.393 ± 0.121 vs. 0.549 ± 0.206 k/g protein) ($P = 0.08$ and $P = 0.07$ for the SOD and the CAT, respectively). The SOD levels and the CAT activities were not significantly different between the prenatal+postnatal, and the postnatal group ($P > 0.05$ for both). The SOD levels and the CAT activities were not significantly different between the postnatal and the control group ($P > 0.05$ for both).

Discussion

Oxidative stress is known to be the cause of tissue toxicity because of various reasons (11,20). ROS including superoxide anion radical, hydroxyl radical, and NO may lead to cellular injury when they are produced in excess or when the antioxidant defense systems are destroyed (21). The main ROS that have

Table. The MDA and NO levels, and the activities of SOD and CAT.

GROUPS	SOD (U/g protein)	CAT (k/g protein)	NO ($\mu\text{mol/g}$ wet tissue)	MDA (nmol/wet tissue)
I - Control (n = 6)	0.071 ± 0.02	0.549 ± 0.206	3.578 ± 1.718	7.288 ± 2.917
II - Prenatal+postnatal (n = 7)	0.053 ± 0.01	0.393 ± 0.121	5.498 ± 1.379	13.941 ± 5.675
III- Postnatal (n = 7)	0.062 ± 0.02	0.455 ± 0.101	3.637 ± 0.891	8.389 ± 2.553
P values				
I-II	n.s.	n.s.	0.02	0.009
I-III	n.s.	n.s.	n.s.	n.s.
II-III	n.s.	n.s.	0.019	0.019

n.s.: non-significant

to be considered are superoxide anion, which is predominantly generated by the mitochondria; hydrogen peroxide (H_2O_2), produced from O_2 by the action of superoxide dismutase (SOD); and peroxynitrite ($ONOO^-$), generated by the reaction of O_2 with NO (11,21). These continuously produced ROS are scavenged by endogenous enzyme activities such as SOD and CAT (22). Under some circumstances, these endogenous antioxidant defenses are likely to be disrupted because of overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately restock antioxidants in tissues (12). It was reported that free radicals generated by EMF effects are thought to cause some illnesses such as cancer (2,23). In addition, studies show that electromagnetic field increases concentrations of free radicals and also increases the possibility that they can damage the body (24,25). We studied oxidative stress effects on developing spinal cord tissues of rat pups by evaluating the MDA, NO levels, and the antioxidant enzyme activities measurement.

NO is formed from L-arginine by nitric oxide synthase (NOS). Inducible NOS (iNOS) is expressed only after exposure to specific stimulants such as cytokines and calcium ionophore in various cells. Under abnormal conditions, iNOS can be expressed in most tissues including endothelial cells, astrocytes, and neurons (25-27). The possible reason for the increased NO levels of the spinal cord in 50 Hz EF exposed the prenatal+postnatal group is increased activities of iNOS, since high tissue NO level in the prenatal+postnatal group when compared to the control group suggests that the NOS activity may increase in such abnormal conditions. In this study, we found that the NO levels spinal cord tissue was significantly higher in the prenatal+postnatal group when compared to the control group. However, NO levels were not significantly different between the postnatal and the control group.

The degree of LPO was assessed according to the MDA formation which was routinely used as a marker of LPO (12). The MDA level, an end product of LPO, significantly increased in the 50 Hz electric field exposed spinal cord of rats. Subsequently, the increased MDA levels were in agreement with the

results of previous studies in plasma, lung, and hepatic tissue (1,3,21,28,29). Thereby, the increased MDA level in the spinal cord suggests that 50 Hz electric field may induce peroxidative reactions in lipids (28,29). SOD catalyzes the conversion of superoxide radicals to H_2O_2 . It protects the cell against the toxic effects of superoxide radicals (4,30). In this study, the SOD and CAT activities insignificantly decreased in the spinal cords of the prenatal+postnatal group. Although the SOD and CAT enzyme levels did not change in the prenatal+postnatal group, there was a prominent increase in the NO and MDA levels. These signs show that oxidative stress can occur with lipid peroxidation except for SOD and CAT ways.

The results of this study were very interesting as NO and MDA levels were significantly higher in the prenatal+postnatal group when compared to both the postnatal and the control groups. However, there was no difference between the postnatal group and the control group in terms of NO and MDA levels and antioxidant enzyme activities. In the prenatal+postnatal group, both SOD and CAT enzyme activities decreased when compared to the control group, but it was not statistically significant. These findings show that exposure to EFs that start before birth and continue until puberty causes oxidative stress in the spinal cord of rat pups. However, exposure to EF after the birth did not change the oxidant/antioxidant parameters in the spinal cord of rats. These results show that embryological developing and transforming spinal cord tissues are more susceptible to EFs. In addition, the findings of this study show that oxidative stress of spinal cord may have occurred because exposed electric field of the prenatal+postnatal group was exposed twice as long as the animals of the postnatal group. These may explain the observed differences but more studies are needed about this issue.

The MDA and NO levels of the prenatal+postnatal group were increased when compared to the postnatal and control groups. This proves that long term EF exposure causes oxidative stress beginning from before birth until puberty. There was no difference in MDA and NO values between the postnatal group and the control group. This situation shows that either rat pups are more sensitive to the electric field in the prenatal term or extending the duration of the

exposure of EF causes oxidative stress in rat spinal cord. Only in the prenatal period, advanced studies on the effect of EF are necessary.

In conclusion, experimentally 50 Hz electric field may cause oxidative stress in the spinal cord of rats in the prenatal+postnatal period.

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