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Research Article

Biochemical Changes in The Intervertebral Discs After Electromagnetic Radiation: An Experimental Study

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Summary

Objective: We investigated the intervertebral discs in a rat electromagnetic radiation (EMR) model to demonstrate that electromagnetic radiation is a cause of degenerative intervertebral discs.
Methods: Rats were randomly divided into four groups: group I consisted of control rats, and groups II-IV comprised electromagnetically irradiated with 900, 1800 and 2400 MHz. The rats were placed into a specially designed mechanism, and their heads were exposed to 900, 1800 and 2400 MHz microwaves irradiation for 1 h per day for 2 months. The control group rats were placed into same system. The same procedure was applied to the control group while the rats were not exposed to the electromagnetic waves.
Results: The intervertebral disc Interleukin-1β levels as well as the total antioxidative capacity (TAC) and total oxidative capacity (TOS) values in the EMR groups (900, 1800, 2400 MHz) were higher than those in the control group ($p<0.05$). In the EMR groups, group II intervertebral disc Interleukin-1β levels and TAC were not significantly different from those in group III ($P>0.05$). Group II intervertebral disc TOS was significantly different from that in groups III and IV ($P<0.05$). In the Oxidative Stress Index (OSI) comparisons , group II was significantly different from groups I, III and IV ($P<0.05$).
Conclusions: Electromagnetic radiation increased the intervertebral disc release of inflammatory cytokine IL-1β and oxidative radicals. This process can lead to degeneration of intervertebral disc.

Key words: Electromagnetic radiation, intervertebral disc degeneration, oxidative stress, Rat

Elektromanyetik Radyasyondan Sonra İntervertebral Disklerdeki Biyokimyasal Değişiklikler: Deneysel Çalışma

Özet

Amaç: Elektromanyetik radyasyonun (EMR) intervertebral disk dejenerasyonunun bir nedeni olabileceğini göstermek için sıçan elektromanyetik radyasyon modelinde intervertebral diskleri araştırdık.
Yöntem ve Gereç: Sıçanlar rastgele dört gruba ayrıldı: grup 1 kontrol grubu, grup 2-4 900,1800 ve 2400 MHz ile elektromanyetik dalgaya maruz kaldılar. Sıçanlar özel olarak dizayn edilmiş düzeneğe yerleştirildi ve kafaları 900,1800 ve 2400 MHz dalgaya 2 ay

boyunca günde 1 saat maruz kaldılar. Kontrol grubu da sisteme aynı şekilde yerleştirildi. Aynı prosedür uygulandı ancak elektromanyetik dalgaya maruz bırakılmadılar.
Bulgular: İntervertebral disk interlökin (IL)-1β düzeyleri, total antioksidan kapasitesi (TAC) ve total oksidativ kapasitesi (TOS) değerleri EMR gruplarında (900, 1800, 2400 MHz), kontrol grubundan daha yüksek bulunmuştur($p < 0.05$). EMR gruplarında; intervertebral disk IL-1β ve TAC düzeyleri açısından grup II ve grup III arasında farklılık yoktu($p > 0.05$). Grup II intervertebral disk TOS düzeyi ise grup III ve IV'e göre anlamlı derecede farklılık mevcuttu($p < 0.05$). Oksidativ steres indeksi (OSI) karşılaştırmalarında; grup II değeri, grup I, III ve IV'den anlamlı derecede farklılık mevcuttu($p < 0.05$).
Sonuç: Elektromanyetik radyasyonun etkisiyle intervertebral diskte inflamatuvar sitokinlerden IL-1β düzeyinin ve oksidatif radikallerin salınımının arttığı görülmektedir. Bu süreçte intervertebral diskte dejenerasyona neden olabilmektedir.

Anahtar Kelimeler: Elektromagnetik radyasyon, intervertebral disk dejenerasyonu, oksidatif stress, sıçan

INTRODUCTION

Intervertebral disc degeneration is considered to be one of the most important factors responsible for lower back pain. Three types of stress in an individual's environment are thought to contribute to disc degeneration: dynamic stress (e.g., hard work, posture, externally caused injury), biochemical stress (e.g., nutrition, blood flow obstacle), and physiological stress (e.g., age)[16]. Oxidative stress, enzymatic and cytokine reaction have been proposed to cause biochemical stress on intervertebral disc degeneration.

Public and scientific awareness of questions about cellular phone safety has been increased greatly in the last few years due to media reports about potential adverse health effects for humans exposed to radiation emitted from cellular phones[2]. Cellular mobile phones and their base stations produce electromagnetic radiation (EMR), and the effects of EMR on the body depend on its frequency and power (14 Repacholi, 2001). Analogue phones use frequencies between 400 and 450 MHz, and digital mobile phones use frequencies between 850, 900 (similarly used in our study), 1850 and 1990 MHz, while microwave ovens use a frequency of 2450 MHz[18]. Electromagnetic radiation (EMR) or radiofrequency fields of cellular mobile phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation, and by changing the antioxidant defense systems of human tissues, thus leading to oxidative stress[18].

Another important factor implicated in disc degeneration is the cytokine reaction. In an investigation of cytokine involvement in smoking-induced changes, cytokine infiltration into intervertebral discs reduced chondrocyte activity via autocrine and paracrine effects in a manner that was peculiar to the inflammatory cytokine[16]. Interleukin-1β (IL-1β) plays a central role in the cytokine network and is one of the inflammatory cytokines that introduces prostaglandin E1, matrix metalloproteinase-3, NO, and other substances to intervertebral discs. These factors cause degeneration of the discs[16]. Oda et al. showed IL-1β levels in discs, demonstrating that cytokines play a role in the development of disc degeneration induced by smoking[16].

We aimed to study the effects of electromagnetic radiation on the degeneration of intervertebral discs by evaluating the effect of electromagnetic radiation on oxidative stress and enzymatic and cytokine reactions in disc tissue.

MATERIAL AND METHODS

Animal Model

Twenty-four male Spraque-Dawley rats (8-week old, 150–200 g) obtained from the

Laboratory Animal Production Unit of Suleyman Demirel University were used in the study. The animals were procured, maintained and used in accordance with the "Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by the Suleyman Demirel University, Animal Ethical Committee". They were kept in an environment of controlled temperature (24–26 ºC), humidity (55–60%), and photoperiod (12 h of light and 12 h of dark) for one week before the start of experiments. A commercially balanced diet (Hasyem Ltd., Isparta, Turkey) and tap water were provided ad libitum.

Experimental Design

The animals were randomly divided into four equal groups (consisting of 6 rats each):

Group I rats were used as the control group (without exposure to EMR); these rats were held in the EMR tube for 60 minutes a day for 60 days (two months) under the same environmental conditions. **EMR groups:** group II rats were exposed to 900 MHz, group III rats were exposed to 1800 MHz, and group IV rats were exposed to 2450 MHz.

All of the rats in the EMR groups were exposed to EMR from the generator for 60 minutes a day for 60 days. The EMR exposure time was from 11:00–12:00 a.m. on each day. At the end of the study, the rats in all of the groups were anesthetized with intraperitoneal administration of ketamine(50 mg/kg)-xylazine (6 mg/kg) after the last dose exposure to EMR. The rats were sacrificed by collecting blood samples from the posterior vena cava. The L1-S1 vertebral column from around the muscles of rats was peeled off and removed. The disc tissues, from L3-S1, were removed because they are the three largest disc tissues and -86°C for biochemical studies.

Exposure device

Radiation for the study (900, 1800, 2450 MHz) was provided by an electromagnetic generator continuously for 60 minutes each day for two months (peak power, 2 W; average power density, 1.04 mW/cm2) (Figure 1). The predicted average specific absorption rate (SAR) value was measured at 1.04 W/kg. The power density measurements were made with an electromagnetic field meter (Holaday Industry Inc.) produced at the electromagnetic compatibility (EMC) laboratory of the School of Electronic Engineering (Suleyman Demirel University, Isparta, Turkey). The exposure system consisted of a plastic tube cage (length: 12 cm, diameter: 5.5 cm) and a dipole antenna. The entire body of each rat was positioned in close contact above the dipole antenna, and the tube was ventilated from head to tail to decrease the stress of the rat while in the tube.

Figure 1: *Figure of Electromagnetic Generator*

Biochemical analysis

Tissue Sampling and Homogenization

On the last day of the study, immediately after the euthanization, the disc tissues of the rats were removed to determine the levels of TAC, TOS, and OSI. Before biochemical assays, all tissues were weighed and placed in empty glass tubes. Each tissue was weighed and diluted in 10 volumes of phosphate buffer (pH 7.4), and then the tissues were homogenized in a motor-driven homogenizer. The homogenate was separated with a refrigerated centrifuge at 4,000 rev/min for 15 min and then placed in labeled vials and stored at -80°C. Microprotein levels were measured using the Lowry method[5].

Measurement of total antioxidative capacity (TAC)

The TAC levels of all tissues were measured using a novel automated colorimetric measurement method developed by Erel[4,5]. In this method, the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction and reacts with the colorless substrate O-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of all of the homogenates, preventing the color change and thereby providing an effective measure of the total antioxidant capacity of the plasma. The assay has excellent precision values, which are lower than 3%. The results are expressed as μmol Trolox equiv./gr protein.

Measurement of total oxidant status (TOS)

TOS levels of all tissues were determined using a novel automated measurement method developed by Erel[4]. In this method, oxidants present in the sample oxidize the ferrous iono-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion turns a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide, and the results are expressed as micromolar hydrogen peroxide equivalents per g protein (μmol H_2O_2 equiv/gr protein).

Oxidative stress index (OSI)

The percent ratio of the TOS to the TAC provides the oxidative stress index (OSI), an indicator of the degree of oxidative stress (4). The OSI value was calculated according to the formula: OSI=[(TOS, μmol H_2O_2 Equiv./gr protein)/(TAC, μmol Trolox equiv./gr protein)].

Interleukin-1β determination

Each tissue weighed in phosphate buffer (pH 7.4) was diluted in 10 volumes, chopped with a tissue chopper (Janke & Kunkel Ultraturrax T-25, Germany) and then sonicated with a Branded sonic homogenizer (UW-2070 Bandeun Electronic, Germany). Tissue samples were separated by centrifugation (Eppendorf 5415-R (Germany) brand refrigerated centrifuge) and 4,000 rev/min for 15 min, and the supernatant from the IL-1β was studied. The supernatants were stored at −20°C until the cytokine assays were performed. Cytokines in the tissue homogenate supernatants were measured using an enzyme-linked immunosorbent assay from commercially available kits and conducted according to the manufacturer's instructions. The assay kits for IL-1β were purchased from RayBio mark (USA).

Statistical analysis

Data were expressed as the mean±standard deviation (SD). Comparisons between

controls and each of the groups were made using the Mann–Whitney non-parametric test for independent samples. Statistical analyses were performed by Statistical Package for the Social Sciences (SPSS) for Windows version 11.5 (SPSS Inc., Chicago, IL, USA) and a $p< 0.05$ was considered significant.

RESULTS

Biochemical analysis

Determination of total antioxidative capacity (TAC), total oxidative status (TOS) and Oxidative Stress Index (OSI):

The determination of TAC, TOS and OSI values by group are shown in Table 1. The intervertebral disc TAC and TOS in the EMR groups were higher than in the control group ($p<0.05$). In the EMR groups, the group II intervertebral disc TAC was not significantly different from that of groups III and IV ($P>0.05$), whereas the group III intervertebral disc TAC was significantly different from that of group IV ($P<0.05$). The group II intervertebral disc TOS was significantly different from that of groups III and IV ($P<0.05$), whereas the group III intervertebral disc TOS was not significantly different from that of group IV ($P>0.05$). For the OSI comparisons, the OSI in group II was significantly different from that of group I, III and IV ($P<0.05$). The group I OSI was not significantly different from that in groups III and group IV ($P>0.05$), and the group III OSI was not significantly different from that in group IV ($P>0.05$).

Measurement of intervertebral disc IL-1β levels

The intervertebral disc IL-1β levels by group are showed in Table 2. The intervertebral disc IL-1β levels in the EMR groups (900, 1800, 2400 MHz) were significantly different from those in the control group ($P<0.05$). In the EMR groups, group II intervertebral disc IL-1β levels were not significantly different from those in group III ($P>0.05$), and group II intervertebral disc IL-1β levels were significantly different from those in group IV ($P<0.05$). Also, group III intervertebral disc IL-1β levels were significantly different from those in group IV ($P<0.05$).

Table 1. The levels of total antioxidative capacity (TAC), total oxidant status (TOS) and oxidant stress index (OSI) in the rat groups.

Parameters	Control	900MHz	1800MHz	2400MHz
TAC(μmolTroloxEqv./ gr protein)	0.08±0.03	0.14±0.02	0.14±0.01	0.17±0.02
TOS(μmol H_2O_2 Eqiv/ gr protein)	1.02±0.25	3.26±0.40	1.98±0.22	1.86±0.28
OSI (AU)	1212.90± 170.81	2247.10± 535.35	1406.84± 210.29	1089.09± 220.88

The value represent the mean±SD
TAC compares control- 900/ 1800/ 2400 MHz (*p*<0.05*),* 900 MHz- 1800/ 2400 MHz (*p>0.05),* 1800-2400 MHz (p<0.05)
TOS compares control- 900/ 1800/ 2400 MHz (*p*<0.05*),* 900 MHz- 1800/ 2400 MHz (*p<0.05),* 1800-2400 MHz (p>0.05)
OSI compares control- 900 MHz (P<0.05), control-1800/2400 MHz (P>0.05), 900 MHz- 1800/ 2400 MHz (*p<0.05),* 1800-2400 MHz (p>0.05) Arbitrary Units (AU)

Table 2. The levels of interleukin-1β were detected in the intervertebral discs of groups of rats. Data represent means ±SD Standard deviation.

Groups	*n*	Means±SD
Control	6	101.36 ± 17.87
900 MHz	6	615.42 ± 123.15
1800 MHz	6	520.56 ± 202.42
2400 MHz	6	221.89 ± 30.91

DISCUSSION

Degenerative disc disease in the lumbar spine can cause significant lower back pain and is an important clinical problem that affects a large portion of the population. EMR has long been known to be detrimental to the human body. However, there is still no definitive proof that EMR causes intervertebral degenerative discs (IVDD). In this study, we sought to determine the relationship between EMR and IVDD by examining intervertebral discs in an EMR rat model.

Mobile phones (MP) and their base stations produce EMR. EMR is absorbed by the body and produces heat, but the body's normal thermoregulatory processes remove this heat. All established health effects of EMR exposure are related to thermal effect. A typical mobile phone operates at a power output of 0.25 W, which results in a specific energy absorption rate of about 1.5 W/kg and an associated very low rise in brain temperature (maximum, 0.1°C)[20]. Thus, the possible biological effects from cellular phone use would not be expected to be thermal in nature.

This study revealed that more biochemical changes have been occured at 900 MHz. Nylund et al.[15] have determined that the 1800 MHZ GSM radiation has very small effect on the proteoma of EA.hy926 (Human endothelial cell line) cell, as compered to 900 MHz GSM radiation. Nylund et al.[15] thought that might be different exposure frequencies (900 MHz vs 1800 MHz) and differences in SAR (Specific absorbtion rate) distribution. In the 900 MHz GSM group there is more non-uniform SAR distrubition than in the 1800MHz group. Also Panagopoulos et al.[19] used a model biological sysytem, the reproductive capacity of Drosophila melanogaster, to compare the biological activity between the 900 MHz and 1800 MHz mobile phone radiation. They found out that both types of radiation decrease significantly and non thermally the insect's reproductive capacity, but GSM 900 MHz seems to be even more bioactive than DCS 1800 MHz. Panagopoulos et al. thought that the difference seems to be dependent mostly on field intensity and less on carrier frequency.

"Hardel et al.[6,7] and Lonn et al.[12] reported that exposure to mobile phone radiation increased the incidence of brain tumors and acoustic neurinoma, depending on the duration of mobile phone use. Belyaev et al.[1] investigated whether exposure of rat brain to microwaves (MWs) of the global system for mobile communication (GSM) induces DNA breaks and changes in chromatin conformation and gene expression. Leszczynski et al.[11] reported that heat shock protein-27 (hsp27) is one of the affected proteins and that mobile phone exposure causes a transient increase in phosphorylation of hsp27. Based on the

known functions of hsp27, they put forward the hypothesis that mobile phone radiation-induced activation of hsp27 may (i) facilitate the development of brain cancer by inhibiting the cytochrome c/caspase-3 apoptotic pathway and (ii) cause an increase in the blood brain barrier permeability through the stabilization of endothelial cell stress fibers. Finally, Dasdag et al. showed that the apoptotic process can be affected by radiofrequency radiation[3]."

Other important factors implicated in disc degeneration include the cytokine reaction. In an investigation of the involvement of cytokines in smoking-induced changes, cytokine infiltration into the intervertebral discs reduced chondrocyte activity via autocrine and paracrine effects that were specific to the inflammatory cytokine. IL-1β, which plays a central role in the cytokine network, is one of the inflammatory cytokines that introduces prostaglandin E1, matrix metalloproteinase-3, NO, and other substances into intervertebral discs[16]. Furthermore, IL-1β induces proteases such as matrix metalloproteinase (MMP), which breaks down collagen and proteoglycan and, in turn, causes intervertebral disc degeneration[14]. In the present study, the IL-1β level in discs was determined and demonstrated that cytokines play a role in the development of disc degeneration induced by EMR.

Radiation is known to induce oxidative stress, which in turn activates the apoptotic pathway[17]. Oxidative stress is a cellular or physiological condition of elevated concentrations of reactive oxygen species that cause molecular damage to vital structures and functions[13]. It has been reported that the signal for apoptosis can be generated by the effect of radiation on cell membranes, apparently through lipid peroxidation[17]. Oxidative stress may be an important factor in the pathophysiology of radiation-mediated effects[18]. Mobile phone-induced free radical formation in other tissues has been reported[8,9,10]. Ilhan et al. demonstrated that mobile phones biochemically cause oxidative damage by increasing the levels of NO and MDA as well as XO and ADA activities in brain tissue in a rat model of exposure to EMR[8]. These continuously produced ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (CAT). Under some circumstances, these endogenous antioxidant defenses are likely to be perturbed due to overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue[18]. The number of different antioxidant components in tissues makes it relatively difficult to measure each antioxidant component separately. In addition, because there is cooperation between various antioxidants, examining one in isolation from the rest may not accurately reflect their combined action[2]. Therefore, the measurement of total antioxidant capacity (TAC) and total oxidant status (TOS) developed by Erel[4,5] seems to represent a suitable biochemical parameter for evaluating the overall oxidant and antioxidant status resulting from antioxidant intake or production and their consumption by the increasing levels of oxidative stress[2].

CONCLUSION

In summary, in the EMR groups, oxidative stress markers and cytokine levels were extremely high compared with the control group. EMR-induced oxidative stress products and cytokines may play a role in disc degeneration. Between the EMR groups, OSI, TOS and IL-1β levels are most affected in group II (900 MHz).

Declaration of Interest:

This study was supported by the Turkish Neurosurgery Society Scientific Research Committee. The authors alone are responsible for the content and writing of the paper.

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