

Effects of 900 and 1800 MHz Electromagnetic Field Application on Electrocardiogram, Nitric Oxide, Total Antioxidant Capacity, Total Oxidant Capacity, Total Protein, Albumin and Globulin Levels in Guinea Pigs

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Summary

The aim of this study was to investigate the effects of 900 and 1800 MHz electromagnetic field (EMF) emitted by base station antennas implementing the mobile telephone communication on electrocardiogram (ECG) and some biochemical parameters. Animal material was consisted of 26 male guinea pigs, weighing 652 ± 22.8 g in average. Animals were divided into three groups, as group 1 (control n=7), group 2 (900 MHz EMF n=10) and group 3 (1800 MHz EMF n=9). Groups 2 and 3 were subjected to EMF for 20 days, daily between 13:00-17:00 pm for 4. Total protein (TP), albumin, globulin, plasma oxidant status (TOS), total antioxidant capacity (TAC) and Nitric Oxide (NO) levels were determined in plasma. Statistical comparison of data obtained from plasma analysis revealed a significant difference in guinea pigs subjected to 900 and 1800 MHz radioactive field for albumin, globulin and TOS levels ($P < 0.05$). NO reflected a significant difference in 900 MHz EMF subjected guinea pigs, compared to the control group ($P < 0.05$), however no differences were observed in 1800 MHz EMF subjected animals. Data obtained from ECG and plasma analysis indicated that EMF subjection may lead to some irregularities in the autonomic control of the heart in addition to oxidative damage.

Keywords: Guinea pig, Electromagnetic field, ECG, Nitric oxide, Total antioxidant capacity, Total oxidant status

Kobaylarda 900 ve 1800 MHz Elektromanyetik Alan Uygulanmasının EKG, Nitrik Oksit, Total Antioksidan Kapasite, Total Oksidan Kapasite, Total Protein, Albumin ve Globulin Düzeyleri Üzerine Etkileri

Özet

Çalışmada mobil telefonların iletişimini gerçekleştiren baz istasyonu antenlerinin yaydığı 900 ve 1800 MHz elektromanyetik alanın (EMF) elektrokardiyogram (ECG) ve bazı biyokimyasal parametreler üzerine etkilerinin araştırılması amaçlandı. Hayvan materyali olarak ortalama ağırlıkları 652 ± 22.8 gr olan 26 erkek kobay kullanıldı. Hayvanlar 1. grup (kontrol n=7), 2. grup (900 MHz EMF n=10) ve 3. grup (1800 MHz EMF n=9) ve olarak 3 gruba ayrıldı. Her 3 grupta birbirlerinin elektromanyetik alanlarından etkilenmeyecek şekilde farklı odalarda barındırıldı. 2. ve 3. gruplara 20 gün süreyle her gün 13:00-17:00 saatleri arasında 4 saat süreyle EMF uygulandı. Çalışma sonunda ECG ve daha sonrada eter anestezisi altında kalpten antikoagulanlı tüplere kan alındı. Alınan kanlar 4°C 1550 g de 10 dakika santrifüj edildikten sonra plazmaları alınarak analiz edilinceye kadar -80°C de saklandı. Alınan ECG kalp atım sayısı, P dalgasının süresi ve amplitüdü, PR aralığı, R dalgasının amplitüdü, S dalgasının süresi, QRS kompleksinin süresi, QT aralığı ve T dalgasının amplitüdü yönünden incelendi. Plazmada ise total protein (TP), Albumin, Globulin, plazma oksidan status (TOS), total antioxidant capacity (TAC) ve Nitrik Oksit (NO) analizleri gerçekleştirildi. Plazma analizlerinden elde edilen sonuçların istatistiksel karşılaştırılması sonucunda TP ve TACTa herhangi bir anlam bulunamazken, Albumin, Globulin ve TOS'ta 900 ve 1800 MHz elektromanyetik alana maruz kalan deneklerde kontrole göre anlamlı değişme tespit edildi ($P < 0.05$). NO ise 900 MHz EMF maruz kalan deneklerde kontrole göre anlam bulunurken ($P < 0.05$) 1800 MHz EMF maruz kalan deneklerde kontrole kıyaslandığında bir farklılık bulunamadı. ECG değerlendirmelerinde ise 2. grupla kontrol grubu arasında QT aralığı, T dalgasının amplitüdü ve kalp atım sayısı yönünden istatistiksel bir anlam ($P < 0.05$) tespit edildi. PR aralığının her iki deneme grubu ile kontrol grubu arasında anlamlı ($P < 0.05$) bir değişim mevcutken QRS süresi sadece 2. deneme grubu ile kontrol grubu arasında anlamlı ($P < 0.05$) bir değişim bulundu.

Anahtar sözcükler: Kobay, Elektromanyetik alan, EKG, Nitrik oksit, Antioksidan kapasite, Oksidan kapasite



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INTRODUCTION

Rapid advances in technology gives increase to the usage of EM waves, hence men is subjected to too much EM waves. This increase in the last years put forward the discussion about the probable harmful effects for human health ^{1,2}. Effects of electromagnetic (EM) fields produced by mobile phones and base stations are related to their frequencies and power ³. EM fields have two different kind of effects on tissues; thermal and chemical effects ⁴. High frequency EM waves have harmful effects due to heat, while long exposition to low frequency EM waves effect synthesis of biomolecules (DNA, RNA and protein), cell division, cancer formation, calcium exchange and linkage at the membrane at tissue and cell surface. Physiologically and biochemically, noted effects in cells and tissues include cell respiratory deficiency, changes in the hormonal response of tissues and cells, reduction in neutrophile population, alterations in carbohydrate, nucleic acid and protein metabolism, structural changes, affected immune response against different antigenes, triggering damage by letting free radicals to circulation unretained ^{3,5-9}.

There are few studies about effects of radiofrequency fields emitted by mobile phones and base stations on cardiovascular system and the very rare studies performed are more about blood pressure, heart beat number and pacemakers ¹⁰⁻¹². Besides, in the majority of the studies reported, effects of either 900 MHz or 1800 MHz EM waves are investigated and there are very few studies comparing both wave lengths.

Endothelium derived NO provides local or systemic vessel muscles to relax, thus contributes to blood flow and pressure balance ¹³.

Antioxidant system intervenes to abolish the harmful effects of free oxygen radicals produced in the organism. Oxidant-antioxidant imbalance ends up with oxidative stress ¹⁴. Oxygen derived superoxide, peroxide, hydroxyl, nitric oxide and other free radicals are very reactive, hence in case of over production they damage the integrity of phospholipids, proteins, and molecules such as DNA and RNA which functions in the membrane unity ¹⁵. In various biologic events, such as cancer, irradiation damage, electromagnetic field, antimicrobial defence, suppuration, photobiologic effects and aging, these free radicals take an important place. Results are DNA base damages, protein oxidation products and lipid peroxidation products which impair cell balance and injure the cell ¹⁴⁻¹⁸.

In this study, comparative investigation of the probable effects of 900 MHz and 1800 MHz EM wave lengths on the guinea pig heart, a tissue with electrical activity, by monitorization of electrocardiographic changes, blood NO level, variations in total oxidant-antioxidant balance was aimed.

MATERIAL and METHODS

Animals and Experimental Procedure

Twenty seven healthy male guinea-pig with a mean body weight of 652 ± 22.8 g were used. Guinea-pigs were obtained from the Veterinary Control and Research Institute Samsun, and the study was approved by the Veterinary Control and Research Institute Samsun Ethics Committee (Date: 20.05.2010 Number: 2). All the stages of study was performed in Veterinary Control and Research Institute Animal Laboratory (Samsun, Turkey). After their clinical health was verified, the animals were housed in a well-ventilated, temperature-controlled ($22 \pm 2^\circ\text{C}$) hygienic room at 60% relative humidity under a 12-h light/dark cycle. Throughout the study, the guinea-pigs were allowed free access to standard rodent laboratory chow (Samsun, Turkey) and tap water *ad libitum* up to the time of sacrifice.

Guinea-pigs were randomly chosen and divided into three groups. Group 1 was the control group (n= 7). Group 2(n=10) was subjected to 900 MHz EMF and Group 3 (n=10) was subjected to 1800 MHz EMF for 20 days (24.05-14.06.2010) and 4 saat hours daily. One of the guinea-pigs died in group 2 during the study. At the end of the 20 days study period, ECG of all the subjects were monitored and then blood samples were drawn by intracardial puncture into tubes including EDTA as an anticoagulant under ether anaesthesia. All blood samples were centrifuged (1550 g, 10 min, $+4^\circ\text{C}$). The separated plasma samples were then stored at -80°C until analysed.

EMF Subjection

In the study, to apply 900 MHz radio frequency signal to group 2, Everest GSM Simulator (900 CW4, Adapazarı, Türkiye) was used ¹⁹. Generator had 850-950 MHz band interval, 4W maximum output power, 2 RF outputting canal and circular polarization high gain specification equal to mobile phone antenna. For 1800 MHz radio frequency signal, applied to the Group 3, Everest GSM Simulator (1800 CW2, Adapazarı, Türkiye) was used. Generator had 1750-1850 MHz band interval, 2 W maximum output power, 2 RF outputting canal and circular polarization high gain spesification equal to mobile phone antenna. EMF measurements were performed with Portable Field Meter (PMM 8053, Costruzioni Elettroniche Centro Misura Radioelettriche Srl., Italy) which can make instant measurements at x,y,z cartesian coordinates and time axis and EP-330 electric area probe which was able to perform measurements at 100 kHz-3 GHz frequency zone and 0.3-300 V/m interval. During emission, guinea pigs were kept at specially designed plexiglass restrainer cages. Air spaces of 2 cm diameter at the top of the restrainer were created in order to reduce the stress of animals in the cage. Equal distribution of electrical area was achieved by placing the dipol antenna in the middle point of restrainer. Antenna

length was approximately 15 cm. Static grounding was comprised by 1mm thick galvanize was placed at the bottom of the restrainer. Output power for group 2 is preferred as 2 W and 1 W for group 3.

Subjected Field

It is quite difficult to measure the Specific Absorption Rate (SAR) value on an exposed biological tissue, directly. The finite-difference time-domain (FDTD) method has been used to compute the SAR values in a simulation environment²⁰. To compute the SAR values, Dielectric permittivity and conductivity values at the certain frequencies have been obtained using data from Italian National Research Council²¹. Local SAR is a parameter widely used by most authors to compare the absorbed energy in different biological tissues, for example, in rats, in humans, etc.²²⁻²⁵. By chosen electrical properties (for 900 MHz and 1800 MHz) from the tables, local SAR values have been calculated with FDTD method. According to calculations σ (S/m) is 1.2298, relative permittivity is 59.893 and local SAR value is 0.1570 W/kg for the heart in 900 MHz group; σ (S/m) is 1.7712, relative permittivity is 56.323 and local SAR value is 1.50 mW/kg for the heart in 1800 MHz group.

Electrical field and power intensity measurements for 6 min over 5 guinea-pigs were obtained. In 4 h total submission, for group 2, electric field was measured between 34.2-36.6 V/m and power intensity was between 3.1-3.55 W/m², and for group 3, grup için electric field was between 22.6-23.8 V/m and power intensity was between 1.35-1.5 W/m².

ECG Procedure

Animals were prepared for ECG without exciting them. ECG electrodes were placed on the skin over *triceps brachii* muscle (*caput longum and caput laterale*) at the front legs and on the skin over *biceps femoris* muscle at the hind legs. Electrode gel was applied on the skin in which electrodes were attached in order to reduce resistance and to obtain a clear record. No anaesthesia was given to the animals during recording. All the ECG's were taken at 1 mV = 10

mm and paper speed was 50 mm/sn. Derivations I, II, III, aVR, aVL and aVF were recorded, but wave durations and amplitude measurements were based on derivation II²⁶.

Biochemical Analysis

NO levels of the specimens were measured colorimetrically (PowerWave XS, BioTek, Instruments, USA) according to the method described by Miranda et al.²⁷ Total oxidant capacity and total antioxidant capacity were measured by commercial kits (Rel Asssay) with the colorimetric method (PowerWave XS, BioTek, Instruments, USA)²⁸. Total protein, albumin and globulin levels were determined with commercial kits (BioMerieux, France).

Statistical Analysis

Descriptive statistics were applied to the obtained data and Duncan was used at the significance control of the difference between the groups²⁹.

RESULTS

Statistical comparison of the data obtained from plasma analysis did not reveal any significance for TP and TAC where a significant differences were concluded for albumin, globulin and TOS between the subjects submitted to 900 and 1800 MHz electromagnetic field and control group (P<0.05). Nitric oxide results reflected a significant variation for 900 MHz electromagnetic field subjected animals compared to the control group (P<0.05), but no difference was observed between the control group and animals subjected to 1800 MHz electromagnetic field (*Table 1*).

Electrocardiographic examination revealed a statistically significant difference between and the control group regarding QT interval, T wave amplitude and heart rate (P<0.05). A significant difference (P<0.05) between group 2 and control group were present in means of PR interval, but a significant difference (P<0.05) for QRS was present only between group 3 and the control group (*Table 2*).

Table 1. Biochemical parameters (mean±SEM) for Group 1 (control n=7) Group 2 (900 MHz EMF n=9) and Group 3 (1800 MHz EMF n=10). Different letters in the same line are statistically significant (P<0.05)

Table 1. Grup 1 (kontrol n=7), Grup 2 (900 MHz n=9) ve Grup 3'e (1800 MHz n=10) ait biyokimyasal parametreler. Aynı satırdaki farklı harfler istatistiksel olarak anlamlı (P<0.05)

Parameters	Group 1 (Control)	Group 2 (900 MHz)	Group 3 (1800 MHz)
Total protein (g/dl)	5.0524±0.13	5.4643±0.20	5.3130±0.08
Albumin (g/dl)	3.9767±0.04 ^a	3.7057±0.09 ^b	3.5699±0.09 ^b
Globulin (g/dl)	1.0757±0.10 ^a	1.7586±0.15 ^b	1.7430±0.08 ^b
TAC (mmolTrolox Equiv./L)	0.9239±0.08	0.9491±0.09	0.8005±0.07
TOS (µmol H ₂ O ₂ Equiv./L)	0.08779±0.01 ^a	0.28631±0.05 ^b	0.34525±0.05 ^b
Nitric oxide (µmol/L)	7.3253±0.45 ^a	9.7931±0.68 ^b	8.8874±0.58 ^{ab}

Table 2. ECG data for Group 1 (control n=7) Group 2 (900 MHz EMF n=9) and Group 3 (1800 MHz EMF n=10). (mean±SEM). Different letters in the same line are statistically significant (P<0.05)

Tablo 2. Grup 1 (kontrol n=7), Grup 2 (900 MHz n=9) ve Grup 3'e (1800 MHz n=10) ait EKG verileri. Aynı satırdaki farklı harfler istatistiksel olarak anlamlı (P<0.05)

Parameters	Group 1 (Control)	Group 2 (900MHz)	Group 3 (1800MHz)
P duration (sc)	0.03286±0.0018	0.03125±0.0013	0.03222±0.0015
PR interval (sc)	0.06857±0.0034 ^a	0.05750±0.0016 ^b	0.05556±0.0018 ^b
P amp. (mV)	0.10143±0.0014	0.1062±0.0062	0.1111±0.0073
R amp. (mV)	0.336±0.047	0.4125±0.030	0.3722±0.028
S wave (sc)	0.0607±0.011	0.0562±0.0062	0.0417±0.0059
QRS duration (sc)	0.039857±0.00014 ^a	0.03875±0.0013 ^{ab}	0.03444±0.0018 ^b
QT interval (sc)	0.1300±0.0038 ^a	0.11375±0.0032 ^b	0.1244±0.0044 ^{ab}
T amp. (mV)	0.0500±0.033	0.0750±0.025	0.0500±0.025
T duration(sc)	0.03286±0.0029	0.03625±0.0018	0.03222±0.0015
Heart beat/min	245.71±2 ^a	282±7.3 ^b	254.9±15 ^{ab}

DISCUSSION

The organism must consistently emit the heat produced at the end of oxidation of nutrients in order to keep body heat steady and makes this with infrared radiation. In other words, remove the body heat with EM waves. At the same time, earth also emits an EM field. EM fields of the living organism and earth are compatible. Advances in technology ruined this consistency, because these instruments that humans use have electromagnetic fields much higher than EM fields of the human body and natural environment.

Most sensitive to EMF influence, are the cardiovascular and nervous systems, particularly the autonomic nervous system responsible, among others, for neurovegetative regulation of the cardiovascular function ³⁰.

Significant increase in the 900 MHz elektromagnetic field emitted group compared to the control group suggests that the hyperthermia caused by the EMF effect ^{31,32} nearby vasodilation ³³ and hypotension ³¹ occurred as a reflex response. Contrary of the data obtained, Kılıçalp et al.¹² reported an important decrease in the heart rate of the guinea-pigs subjected to mobile phones. ECG examination reflected a statistically significant decrease in PR interval, QRS duration and QT interval, in the study groups. Decrease in these intervals is thought as a natural result of the increased heart rate.

Endothelium derived NO is an important indicator of the basal vascular tonus. NO regulates the systemic circulation and also contributes to the local circulations and regulation of the peripheral resistance of the organs such as heart, liver and brain ³⁴. Changes in the arterial pressure and frictional stress with the increased blood

flow in the vessels are the major stimulator of NO release ³⁵. NOS inhibition, which allows NO production increases blood pressure and heart rate ³⁶.

In this study, heart rates of both groups increased when compared to the control group, but only in 900 MHz EMF submitted group, the increase was statistically significant (P<0.05), while it did not show a statistical significance in the group submitted to 1800 MHz EMF. In paralel, NO level showed a significant increase (P<0.05) in the 900 MHz EMF submitted group, compared to the control group, but not in the 1800 MHz EMF submitted group. Plasma NO level elevation in the 900 MHz EMF submitted group may suggest a response to the friction stress with elevated blood flow due to increased heart beat, because endothelium derived NO relaxes vessel smooth muscles and helps blood flow and pressure balance, so its vasodilator effects constitutes a major defence mechanism of the myocardium ¹³.

In previous studies, oxidative stress was determined only by lipid peroxidation and NO as the plasma oxidation indicator, where the antioxidant capacity was monitored by enzyme activities such as plasma or in erythrocyte SOD, CAT, GSH-Px and GSH level ³⁷⁻³⁹ Direct measurement of free radicals in the organism is difficult because of their transient nature and the complexity of available techniques. Since the separate measurements of different oxidant and antioxidant molecules were not practical, measurement of the TOS and TAC has been suggested ^{40,41}. Unlike the other studies in this study both total oxidant status and total antioxidant capacity was measured either at 900 MHz or at 1800 MHz EM fields.

In this study, TOS and TAC were investigated in order to determine oxidative stress. Plasma TOS levels indicated a significant increase (P<0.05) in both groups, compared to the control, however TAC levels did not reflect any

significant increase. Oxidant-antioxidant imbalance yields to oxidative stress¹⁴. In the present study, it was observed that plasma TOS and TAC balance was impaired. This observation indicates that 900 MHz and 1800 MHz EM field subjection will lead to oxidative stress.

Albumin and globulin are two of the most important proteins of the plasma. Plasma albumin constitutes colloid osmotic pressure and at the same time is one of the antioxidants of the plasma. Globulins are in plasma inflammation and immunology parameters. Main target of plasma protein oxidation is albumin⁴². Due to the oxidation of albumin, an important plasma antioxidant, plasma antioxidant defence reduces and constitutes oxidative stress induced tissue injury together with cardiovascular disease risk in these groups of patients⁴³. In this study plasma albumin level in both groups decreased significantly in both groups compared to the control group, where globulin level reflected a significant increase in both groups ($P < 0.05$). No statistically significant changes occurred for total protein and this can be attributed to albumin-globulin ratios balancing each other. Significant decrease in the albumin level may be due to the oxidation of albumin because of the elevation in plasma TOS level and this may constitute a risk for induced tissue injury and cardiovascular diseases⁴³.

In conclusion, changes in rhythm and wave morphologies of the heart due to electromagnetic field submission suggests a likelihood of autonomic system (sympathetic and parasympathetic) to cause various irregularities in the stimulus transmission of the heart. In addition, it can be indicated that the imbalance between TOS and TAC can lead to oxidative injury. It is considered that animals in 1800 MHz EMF group were exposed to a lower wavelength than animals in 900 MHz EMF group because the frequency and wavelength of electromagnetic field are inversely proportional.

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