Melatonin protects the formation of lipid peroxidation induced by 27.17 mhz radiofrequency radiation in rat lung cells and erythrocytes

Ünal Şahin*, H.Ahmet Bircan*, Selçuk Çömlekçi**, M.Fehmi Özgüner***, Ahmet Akkaya*

* Suleyman Demirel University Faculty of Medicine, Department of Chest Diseases, Isparta
** Suleyman Demirel University, Department of Electronic Engineering, Isparta
*** Suleyman Demirel University Faculty of Medicine, Department of Physiology, Isparta

Özet

Melatoninin rat akciğer hücreleri ve eritrositlerinde 27.17 mhz radyofrekans radyasyonu ile oluşturulan lipid peroksidasyonu üzerine önleyici etkisi

Giriş: Radyofrekans radyasyonuna maruz kalan küçük memelilerde melatonin sekresyonunda bir azalma gözlemlenmiştir. Radyofrekans elektromanyetik alanlar yayan 27.17 MHz band radyoların artan kullanımının sağlık üzerine olası etkileri hakkında endişeler olması sebebiyle bu alanların lipid peroksidasyonunun bir belirteci olarak yaygın kullanılan malondialdehid (MDA) düzeyinde değişiklik yapıp yapmadığını araştırdık. Amaç: Bu deneyde direkt manyetik alan (MA) akımına maruz kalan ratların akciğer hücrelerinde lipid peroksidasyonun oluşup oluşmadığını ve manyetik alanların akciğer dokusu üzerindeki etkilerinin melatonin tedavisiyle engellenip engellenmediği belirlemek. Gereç ve yöntem: Çalışmaya otuz rat alındı ve hayvanlar üç gruba (MA, MA+melatonin, kontrol) eşit olarak dağıtıldı. MA+Melatonin grubuna günlük 4mg/kg intraperitoneal melatonin enjekte edildi ve uyarlanmış anten impedansının kör-yükü 50 Ohm olan 27.17 MHz manyetik alana iki hafta boyunca, haftada altı gün, günde 2.5 saat maruz bırakıldı. MA grubu da aynı manyetik alana aynı süre maruz bırakıldı. Kontrol grubuna ise sadece 1 mg/kg fizyolojik salin solüsyonu intraperitoneal uygulandı. Deney sonunda tüm ratların toraks boşlukları açıldı. Kardiyak ponksiyon ile kan örnekleri alındı, akciğerler çıkarılarak homojenize edildi ve her üç grupta MDA düzeyleri çalışıldı. Bulgular: MDA düzeyleri eritrosit hemolizatında 3.53 0.64 nmol/ml, 1.72 0.64 nmol/ml (p=0.000) ve akciğer dokusunda 2.72 0.51 nmol/gr, 1.44 0.28 nmol/gr (p=0.000) olmak üzere MA grubunda MA+melatonin grubuna göre anlamlı olarak yüksek bulundu. Sonuç: Melatonin ratlarda MA maruziyetiyle oluşturulan oksidatif hasara karşı eritrositler ve akciğer hücreleri için anlamlı bir koruma sağlamaktadır.

Anahtar Kelimeler: Oksidatif hasar, melatonin, elektromanyetik alan, serbest radikaller, akciğer.

Abstract

Introduction: A decrease in melatonin secretion has been observed in small mammals under exposure to radiofrequency radiation. As there is some concern about possible health effects of the increasing use of 27.17 MHz citizen band radios emitting radiofrequency electromagnetic fields, we examined whether such fields would alter the level of malondialdehyde (MDA) that is extensively used as a marker of lipid peroxidation. Objective: The purpose of this experiment was to determine whether the exposure of rats to pulsed direct current magnetic fields (MFs) would cause a lipid peroxidation in the rat lung cells. Also, the experiment was carried out to investigate whether treatment with melatonin could block the effect of magnetic fields on lung tissue. Material and methods:Thirty rats were included in the study. The animals were divided into three groups (MF and MF+melatonin, controls) equally. The animals in the MF+melatonin group were injected with melatonin (4mg/kg, intraperitoneal) and exposed to a 27.17 MHz MFs, dummy-load was 50 Ohms for matched antennas impedance. Duration of exposure was 2.5 hours/day, 6days/week for two weeks. MF group were exposed to same magnetic fields at the same times. Control rats were injected with only physiologic saline solution (1mg/kg, intraperitoneal). After the experiment, the rats were sacrificed and thoracic cavity was explored; blood samples were obtained from the cardiac region and lungs were extracted and homogenised. MDA levels were studied in all groups. Results: MDA levels were significantly higher in the MF group than the MF+melatonin group, for erythrocyte hemolysate 3.53 0.64 nmol/ml vs 1.72 0.64 nmol/ml (p=0.000), and for lung tissue 2.72 0.51 nmol/gr vs 1.44 0.28 nmol/gr (p=0.000), respectively. Conclusion: Melatonin provides significant protection for lung cells and erythrocytes from oxidative damage induced by MFs exposure.

Keywords: Oxidative damage, Melatonin, electromagnetic field, free radicals, lung.

Yazışma Adresi:
Yrd.Doç.Dr. Ünal Şahin
SDÜ Tıp Fakültesi Göğüs Hastalıkları AD, Isparta
Tel : 0246 2112423
E-mail: drunsahin@yahoo.com

Introduction

The interaction of radiofrequency (RF) radiation with living systems, including human beings, is a complex function of many parameters. The electrical proper-

ties of the living system and its geometry determine the amount of radiation reflected, transmitted and absorbed for a given exposure field. The exposure field is characterised by the frequency, intensity, polarisation and type or, a plane wave, leakage field, the near-field or far-field (1).

For human beings maximum energy absorption takes place between 30 and 100 MHz, depending on the body size and the environment. The specific absorption rate (SAR) depends on the following: (a) The incident field parameters; intensity, polarisation and source-object configuration (far-and near-field), (b) The characteristics of the exposed body; the size, external and internal geometry, dielectric properties of the various tissue layers in an inhomogeneous multi-layered object (such as a human or animal body), (c) Ground effects and reflector effects of other objects in the field, such as metal surface near the exposed body (2).

In the view of widespread use of 27.12 MHz citizen band radios, it is important to look for possible adverse effects of the emitted radiofrequency electromagnetic radiation. Several epidemiological studies have suggested that there may be a relationship between exposure to magnetic fields (MFs) and the increasing incidence of cancer and several disorders (3-6). In the study of MFs in houses, the most widely reported finding is the possible association between MFs exposure and an increased incidence of childhood leukaemia (7).

Recently, an increase was determined in DNA single and double-strand breaks in the brain cells of rats exposed to MFs (8). The mechanisms by which MFs cause damaging effects in the tissues are not well known. Perhaps, free radicals may have a role.

Cellular damage mediated by oxidising lipids has been reported by different studies. It has been shown that lipid peroxidation induced via active oxygen species is involved in the pathogenesis of acute tissue injuries induced by ethanol, haloalkanes, ischemia-reperfusion. Oxidising lipids possess the potential for amplifying free radical initiations and broadcasting oxidation stress to tissue sites distant from the original reaction (9-11). Assessment of MDA has been extensively used as a marker of lipid peroxidation. In the study, we used MDA levels were determined in the hemolysate of erythrocytes and lung tissue homogenates as a criteria for oxidative stress-induced injury.

Melatonin has been reported to be a free radical scavenger (9). Suppression of the blood and pineal gland of the rat or hamster as a consequence of MFs exposure has been reported by several laboratories. In the current series of these studies, a suppression of serum melatonin sometimes occurred without any apparent change in the synthesis of this indolamine within the pineal gland (10). It has also been shown that when animals and tissues are subjected to lipid peroxidation, melatonin causes a substantial protection against the oxidative destruction of lipids (11, 12). In addition, an advantage of melatonin is that it can readily pass through the cells, nuclear membranes and blood-brain barrier.

In the present study, the effect of radiofrequency radiation (27.17 MHz) on lipid peroxidation has been examined in the MFs-exposed rats and possible preventive effect of melatonin on this oxidative damage has been questioned.

Materials and Methods

Thirty albino young male Wistar strain rats, weighing 120-140 g and 6 weeks-old, were used in the study. Animals were housed at room temperature (23 1 oC) and maintained on a 12:12 h light-dark cycle with food and water ad libitum. They were divided into 3 groups.

Control group (C) (n: 10)

The animals in control group received only the same quantity of serum physiologic and were subjected the same environmental conditions as the MFs exposed animals. The control group also had daily intraperitoneal injections of saline (0.1 ml / 100g) containing 5% ethanol for two weeks.

Magnetic field exposed group (MF) (n: 10)

Only MFs exposed group had daily intraperitoneal injections of physiologic saline (0.1 ml / 100g) containing 5% ethanol for two weeks.

Magnetic field exposed and melatonin treated group (MF + m) (n: 10)

Melatonin (Sigma Chemical Co.) was dissolved in ethanol with further dilution in physiological saline (final concentration: 5%). Exposure was 2.5 hours/day, 6 days/week for two weeks. The MFs exposed animals had intraperitoneal single dose of 4 mg/kg melatonin at 10:00 o'clock daily for two weeks following MFs exposure.

Measurement of MDA

According to this method, thiobarbituric acid reactive substance (TBARS) concentrations were determined in tissue homogenates. 3 ml 1% H3PO4 and 0.6% TBA solution were mixed with homogenates. The mixture was kept at hot water for 45 minutes. Then, it was cooled with normal water, 4 ml n-butanol added and vortexed. Absorbance was measured at 532 nm (e=1.56.105 M-1 cm-1) on the spectrophotometer (Schimadzu 1208 UV-VIS, Japan). The results were expressed as nmol/gr protein.

Magnetic fields exposure

Commercial citizen band (CB) hand-held portable transceiver was used, (MIDLAND, USA, Labelled of 4 Watts, 40 Channel). Each rat was irradiated separately in a cage. MFs energy at 27.17 MHz passes through the cage. The cage of animals has been made of only wood. In order to avoid the reflections of RF fields, metallic objects were not used. The size of wooden cage was very critical. To obtain nearly the same value of electromagnetic (EM) field on every point of cage we had to use minimum area. The optimum size was 40x40x30cm.

First, we made by-pass the push-to talk switch of transceiver, in order to use as RF source in test set up. On first channel, RF power on dummy load connected antenna connector was measured. Dummy-load had been 50 ohms for matched antenna impedance. This channel frequency has been measured 27.17 MHz with frequency counter.

Experiment test set-up has been prepared for power and dosimeter calibration. A monopole magnet whip antenna has been used radiating element. A power-meter, Standing Wave Ratio Meter (S.W.R), and a spectrum analyser with loop probe antenna for calibration have been used.

Statistical average of thirty eight consecutive measures performed on individual cages was used to obtain average power density (APD) of each cage. The APD is obtained as 14.47mW/cm2. According to The International Radiation Protection Association (IRPA) exposure standards; for 27 MHz, for 6 min., for general public health and occupational exposure limits are 0.2mW/cm2, 1mW/cm2 respectively (2). In this study; our exposure is always over the recommended limit.

At the end of study, all of the 30 rats had a surgically operations under ether anaesthesia to open the chest area and were obtained blood sample from the cardiac region of heart by 22 G needle syringes. Intracardiac blood samples were centrifuged for 10 minutes at 3000 g for separation of serum. During the operations the tissue samples were taken from lungs of each animal.

Statistical analyses

Statistical analyses were performed with a statistical software package (SPSS for Windows 9.05). All values are expressed as mean±SDs. Inter group comparisons were done using non-parametric Kruskal-Wallis and Mann-Whitney U tests. Values of $p<0.05$ were considered significant.

Results

MDA levels were found to be significantly increased in MF exposed rats (p=0.000, Table 1.) We determined that melatonin treatment decreased the MF induced MDA increase in erythrocyte hemolysate and the lung cells as compared to MF exposed rats.

Table 1 : MDA levels in the groups

	MF †	MF+Melatonin §	Control
Erythrocyte hemolysate (nmol/ml)	3.53±0.64*	1.72±0.64	1.67±0.19
Lung tissue (nmol/gr)	2.72±0.51*	1.44±0.28	1.50±0.42

MDA levels were found significantly different between the groups with Kruskal-Wallis (Erythrocyte hemolysate MDA: X2 = 19.42, p=0.000, Tissue MDA: X2= 19.54, p=0.000) and and Mann-Whitney U tests (Table 2.).

Table 2 : Statistical analysis of the groups with Mann-Whitney U test

	MF † vs MF+Melatonin §	MF † vs Control	MF+Melatonin § vs Control
Erythrocyte hemolysate	Z= -3.78, p=0.000	Z=-3.78, p=0.000	Z= -0.341, p=0.733
Lung tissue	Z= -3.78, p=0.000	Z=3.78, p=0.000	Z= -0.606, p=0.545

Discussion

In recent years some people expressed concern about possible health effects of electric and magnetic fields produced by exposure to extremely low frequency bands. Whether electromagnetic fields are sources of illness in man remains a matter of dispute. The several epidemiological studies that have been performed on human populations are inconclusive (13).

Although weak evidence exists, it fails to support an effect of RF exposure on mutagenesis or cancer initiation. Most laboratory evidence indicates that low-level RF fields are not mutagenic and are unlikely to act as an initiator of carcinogenesis (14-17).

In contrast to the evidence given above, several rodent studies indicate that RF fields may affect DNA directly. When mice were exposed to 2.45 GHz-fields at 10 W/m2 (SAR 1.18 W/kg) for 2 h/day for 120, 150, and 200 days, there was an indication of structural genomic rearrangement in brain and testes cells (18).

Several reports have shown that exposure of rodents to ELF magnetic fields reduces the production, secretion, or both of melatonin from pineal gland (19).

Stevens (20) proposed a relationship between exposure to extremely low frequency (ELF) fields and carcinogenesis through RF fields action on melatonin secretion.

Melatonin, because it is a potent antioxidant, may provide significant protection against MFs induced

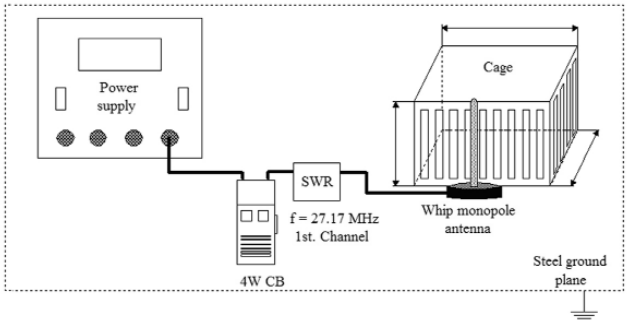

Figure 1: Experimental set-up

free radical damage. Reduction of melatonin increases cells' vulnerability to alteration by carcinogenic agents. Thus, in fact artificial electromagnetic field exposure increases the incidence of cancer in humans. Data from the earlier studies confirm our previous finding that acute MFs exposure causes an increase lipid peroxidation in brain cell of the rat (4, 8, 21).

In the present study melatonin administrated at pharmacological doses was found to prevent the lipid peroxidation in erythrocytes and lung cells in rats induced by 27.17 MHz MFs exposure. The results are similar to those of investigators for rats irradiated 27.12 MHz (13, 22). Treatment of rats with melatonin blocked the effect of MFs exposure.

Because melatonin is a direct free radical scavenger, the drop in serum melatonin could theoretically be explained by an increased uptake of melatonin by tissues that were experiencing augmenting levels of free radicals as a consequence of MFs exposure (20).

In conclusion, the results obtained in the study indicate that melatonin administration at pharmacological doses may have a therapeutic effect on MFs induced erythrocyte and lung cell injury in rats, possibly through its antioxidant action. However, further investigation is required to clarify the detailed mechanism of the effect of melatonin on MFs induced damage.

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