

ORIGINAL ARTICLE

Oxidative Damage in the Kidney Induced by 900-MHz-Emitted Mobile Phone: Protection by Melatonin

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Background. The mobile phones emitting 900-MHz electromagnetic radiation (EMR) may be mainly absorbed by kidneys because they are often carried in belts. Melatonin, the chief secretory product of the pineal gland, was recently found to be a potent free radical scavenger and antioxidant. The aim of this study was to examine 900-MHz mobile phone-induced oxidative stress that promotes production of reactive oxygen species (ROS) on renal tubular damage and the role of melatonin on kidney tissue against possible oxidative damage in rats.

Methods. The animals were randomly grouped as follows: 1) sham-operated control group and 2) study groups: i) 900-MHz EMR exposed (30 min/day for 10 days) group and ii) 900-MHz EMR exposed + melatonin (100 $\mu\text{g kg}^{-1}$ s.c. before the daily EMR exposure) treated group. Malondialdehyde (MDA), an index of lipid peroxidation, and urine *N*-acetyl- β -D-glucosaminidase (NAG), a marker of renal tubular damage were used as markers of oxidative stress-induced renal impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status.

Results. In the EMR-exposed group, while tissue MDA and urine NAG levels increased, SOD, CAT, and GSH-Px activities were reduced. Melatonin treatment reversed these effects as well. In this study, the increase in MDA levels of renal tissue and in urine NAG and also the decrease in renal SOD, CAT, GSH-Px activities demonstrated the role of oxidative mechanism induced by 900-MHz mobile phone exposure, and melatonin, via its free radical scavenging and antioxidant properties, ameliorated oxidative tissue injury in rat kidney.

Conclusions. These results show that melatonin may exhibit a protective effect on mobile phone-induced renal impairment in rats. © 2005 IMSS. Published by Elsevier Inc.

Key Words: Melatonin, Kidney, Mobile phone, Oxidative stress.

Introduction

There is accumulating evidence that exposure to the radio frequency radiation from mobile telephones or their base station could affect people's health (1). Herein if there is any impact on health from mobile telephones, it will affect almost everyone in the world (2–4). The use of cellular phones in recent years has raised many questions about

their safety, because the operator is exposed to electromagnetic (radio frequency) radiation (EMR) in the ultra-high-frequency range (i.e., 300–3000 MHz), the effect of which on the body depends on its frequency and power. There has been increasing interest in the biological effects and possible health outcomes of weak, high-frequency electric and magnetic fields (5). Some studies on magnetic fields and cancer, reproduction and neurobehavioral reactions have reported that different system diseases are related to EMR such as mobile phones (6–9).

Mobile phone-induced free radical formation in other tissues has been reported (10–12). According to these authors, biological systems may interact resonantly with EMR,

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but there is as yet no robust evidence to support this suggestion. Reactive oxygen species (ROS) have been implicated in tissue injury. The main ROS that have to be considered are superoxide anion (O_2^-), which is predominantly generated by the mitochondria; hydrogen peroxide (H_2O_2) produced from O_2 by the action of superoxide dismutase (SOD), and peroxy-nitrite ($ONOO^-$), generated by the reaction of O_2 with nitric oxide (NO). ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (CAT).

The kidney is a major potential route for the absorption of hazardous materials encountered in the environment (10). The mobile telephone emitting 900 MHz radiation may be absorbed by kidney more than other internal organs because mobile phones are often carried in belts. Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress-mediated lipid peroxidation (LPO) in renal tissue and other organs (13). Levels of these endogenous indices of oxidative stress have not yet been reported in EMR-exposed animals.

The lysosomal enzyme NAG is one of the markers of tubular damage most commonly referred to, primarily because NAG assays are sensitive enough to allow dilution of the urine, overcoming any enzyme inhibition (14). Also, NAG excretion has been used as an early and sensitive marker of renal injury and toxicity (15).

Melatonin, a pineal hormone, produced and almost exclusively synthesized and secreted at night, is a very potent and efficient endogenous free radical scavenger. Furthermore, melatonin stimulates the activity or gene expression of several important endogenous antioxidant enzymes, including GSH-Px and SOD. It protects molecules from oxidative damage by stimulating GSH-Px activity, which metabolizes hydrogen peroxide to H_2O (16). The marked protective effects of melatonin against oxidative stress are aided by its ability to cross all biological membranes (17–19).

The aim of the current study is to investigate the possible harmful effects of 900 MHz EMR on kidney and its treatment with melatonin.

Materials and Methods

Animal model. Male Wistar Albino rats (8 weeks old, 150 ng) obtained from the Laboratory Animal Production Unit of Suleyman Demirel University were used in the study. The animals involved in this study were 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°C), humidity (55–60%), and controlled photoperiod (12 h/12 h of light and dark) for 1 week before the start of the experiment. A commercial balanced diet (Hasyem Ltd., Isparta, Turkey) and tap water were provided ad libitum.

Experimental design. Animals were randomly grouped as follows: 1) sham-operated control group ($n = 8$) and 2) study groups: i) 900-MHz EMR exposed (30 min/day for 10 days) group ($n = 8$) and ii) 900-MHz EMR exposed + melatonin treated group ($n = 8$). Melatonin (Sigma Chemical Co., St. Louis, MO, USA) was injected s.c. daily at 17:00 hr with a dose of $100 \mu\text{g/kg}^{-1}$ dissolved in 1:90 ethanol/saline.

Before EMR exposure, animals were anesthetized with sodium pentobarbital, 40–60 mg/kg. A 900-MHz EMR was applied to study groups for 10 days (1/2 h/day, at 5 PM daily). Sham-operated rats (Group I) received isotonic saline solution (an equal volume of melatonin) and were also anesthetized and placed in the tube with the same environmental room conditions as the exposure groups but without exposure to EMR (exposure device off).

At the end of the study, rats were sacrificed and the kidneys dissected out. One gram of kidney tissue was homogenized in a motor-driven tissue homogenizer with phosphate buffer (pH 7.4). Unbroken cells, cell debris and nuclei were sedimented at 2000 g for 10 min, and the supernatant was pipetted into plastic tubes and stored at -70°C until assayed.

Twenty-four-hour urine samples were collected throughout the study, centrifuged for 5 min at 1500 g and the clear supernatants stored at 4°C prior to analysis. All assays were carried out on the day of collection.

Exposure device. A 900-MHz continuous wave electromagnetic energy generator (2 W peak powers, average power density 1.04 mW/cm^2) was used in the study. The power density measurements were made using electromagnetic field meter (Holaday Industries Inc., UK), which was produced at the Electromagnetic Compatibility (EMC) Laboratory of Electronic Engineering Faculty (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 whole bodies of the rats were 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.

Determination of MDA. MDA levels were estimated by the double heating method of Draper and Hadley (20). The principle of the 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^{-1} 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 in tap water, the tubes were centrifuged at 1000 g for 10 min and 2 mL of the supernatant was added to 1 mL of 6.7 g L^{-1} TBA solution in a test tube and the 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 concentration of MDA was calculated by the absorbance

coefficient of the MDA–TBA complex (absorbance coefficient = $1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$) and is expressed as nanomoles/gram (nM g^{-1}) wet tissue.

Determination of urine NAG. Urine NAG was measured according to the method of Yakata et al. at 580 nm as 3-cresol sulfonphthalein released from 3-cresol sulfonphthaleinyl β -D-glucosaminide (21). Creatinine in urine was measured using standard spectrophotometric methods (autoanalyzer, Abbott Aeroset, Chicago, IL, USA) in the hospital clinical laboratory. Urinary NAG excretion was expressed as units per gram (Ug^{-1}) of urinary creatinine to rule out the influence of urinary dilution or concentration.

Determination of SOD activity. Total (Cu–Zn and Mn) SOD activity was determined according to the method of Sun et al. (22) with a slight modification by Durak et al. (23). The principle of the method is based briefly on the inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine/xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 mL ethanol/chloroform mixture (5/3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. Activity was expressed as units per gram (Ug^{-1}) protein.

Determination of GSH-Px activity. Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine (24). The enzymatic reaction in the tube that contained reduced nicotinamide adenine dinucleotide phosphate, reduced glutathione, sodium azide and glutathione reductase was initiated by the addition of hydrogen peroxide (H_2O_2) and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was given in units per gram (Ug^{-1}) protein.

Determination of CAT activity. CAT activity was measured according to the method of Aebi (25). 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. Activities were expressed as k (rate constant) per gram (k g^{-1}) protein. Kidney protein was determined by the Lowry method (26).

Statistical analysis. Data were presented as means $\pm$ SD. A computer program (SPSS 9.0, SPSS Inc. Chicago, IL) was used for statistical analysis. A one-way ANOVA test was applied to data to detect significant differences initially. At the second step, Tukey's post-hoc test was used to compare the groups. Differences were considered significant at $p < 0.05$.

Results

In the EMR-exposed group II, exposure to mobile phone device produced a significant decrease in activities of antioxidant enzymes (CAT, SOD and GSH-Px) in renal tissue

($p < 0.001$) while tissue level of MDA and urine NAG activity in EMR exposed group were higher than those of the control group ($p < 0.001$, Table 1, Figure 1). However, melatonin administration in group III increased activities of antioxidant enzymes (shows antioxidant status) and reduced MDA level (shows LPO degree) and urine NAG activity (shows tubular impairment degree) significantly compared with the EMR group ($p < 0.001$, Table 1, Figure 2). The results are summarized in Tables 1 and 2 and Figures 1 and 2.

Discussion

The use of mobile phones is currently one of the fastest growing technological developments. The close proximity of the antenna of such a device to the abdominal organs has raised concerns about the biological interactions between EMR and the kidney. The direct biological effects of exposure to 900-MHz EMR have not been studied extensively. The 900-MHz mobile phones are mainly used in Turkey and in many other countries.

The duration period for mobile phone use and its frequency are important factors, but the exact duration differs from individual to individual. We use the estimated average time of analogue like mobile phone device for an individual in a day as half an hour. Frequency is the rate at which electromagnetic fields change direction and is measured in Hertz (Hz). One megahertz (MHz) is one million cycles per second. Analogue telephones use frequencies between 800 and 900 MHz, and digital telephones use frequencies between 1850 and 1990 MHz, whereas microwave ovens use a frequency of 2450 MHz. Today's mobile telephones, with a total power output of about 2 W, are estimated to produce insignificant local heating, which is unlikely to produce any deleterious effects (10–12). Recent research from many countries suggests, however, that there are 'non-thermal' effects on living tissue, ranging from changes in the permeability of the blood–brain barrier to changes in encephalogram and blood pressure (27).

The results of *in vivo* studies suggested that melatonin might play a regulatory role, affecting urine output and

Table 1. Mean activities of antioxidant enzymes in kidney homogenates of rats

Groups	CAT (k g^{-1} protein)	SOD (Ug^{-1} protein)	GSH-Px (Ug^{-1} protein)
Control ($n = 8$)	1.61 ± 0.20	0.16 ± 0.03	43.53 ± 6.49
EMR ($n = 8$)	1.23 ± 0.23	0.12 ± 0.02	35.45 ± 5.02
Mel + EMR ($n = 8$)	1.57 ± 0.26	0.15 ± 0.04	51.27 ± 6.94
<i>p</i> value			
I–II	<0.001	<0.01	<0.01
I–III	NS	NS	<0.05
II–III	<0.01	<0.01	<0.0001

Values are given as the mean $\pm$ SD.

NS, not significant; Mel, melatonin.

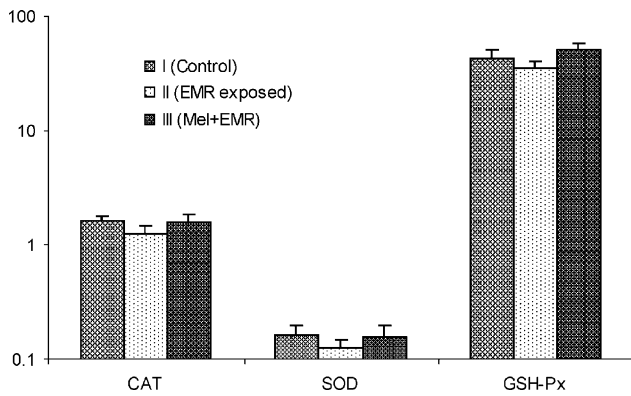


Figure 1. Activities of antioxidant enzymes in kidney homogenates of rats in control, EMR exposed and Mel + EMR groups.

cation excretion (28,29). Localizations of specific melatonin receptors (MR) have been found in renal tissue (30). This finding is highly suggestive of a direct renal functional role for melatonin. Thus, the presence in renal tissue of specific MR has strong biological implications, suggesting that the cellular action of melatonin is affected through membrane transduction and intracellular signaling following melatonin binding to its receptor.

The present study has shown that exposure to EMR with a frequency of 900 MHz had a significant effect on rat kidney, suggesting that ROS were generated under the experimental conditions employed. A significant increase was observed in MDA and NAG levels in the exposed group. The change in activities of antioxidant enzymes with MDA and NAG levels may be regarded as an indicator of increased ROS production occurring during the exposure period and may reflect the pathophysiological process of the exposure.

NAG is a lysosomal enzyme present in high concentrations in the renal proximal tubular cells. NAG is also found in serum but its molecular weight of 140 kDa does not permit glomerular filtration; thus, increased urinary NAG activity is one of the most sensitive markers of renal tubular damage (15,31–33). It appears in the urine after damage of the cell membranes of the proximal tubules and predominantly reflects tubulointerstitial injury and toxicity including

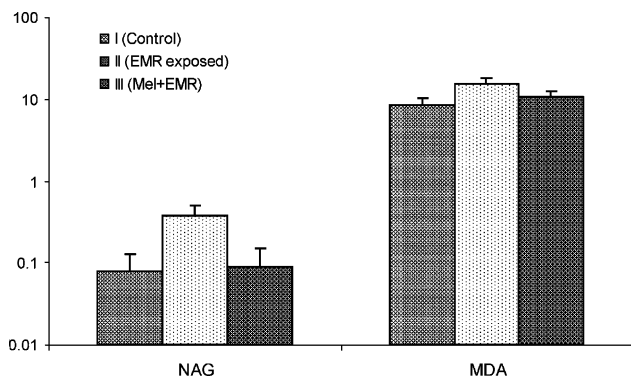


Figure 2. Levels of urine NAG and MDA in kidney homogenates of rats in control, EMR exposed and Mel + EMR groups.

Table 2. Mean NAG activity and levels of MDA in kidney homogenates of rats

Groups	NAG (Ug ⁻¹ creatinine)	MDA (nM g ⁻¹ wet tissue)
Control (n = 8)	0.08 ± 0.05	8.32 ± 1.83
EMR (n = 8)	0.37 ± 0.12	15.43 ± 2.89
Mel + EMR (n = 8)	0.09 ± 0.06	10.91 ± 1.78
p value		
I–II	<0.0001	<0.0001
I–III	NS	<0.01
II–III	<0.0001	<0.001

Values are given as the mean ± SD.

NS, not significant; Mel, melatonin.

those caused by drug toxicity (31,34). To investigate the possibility of subtle renal impairment, we measured urinary NAG activity in EMR-exposed rats. The mean urinary NAG ratio was higher in the EMR-exposed group than in the control group regarding renal impairment. Increased urinary NAG levels in the rats may help us to detect subtle and early alterations in the kidney integrity. Thus, impaired oxidant/antioxidant balance in kidney might be partially responsible for the adverse renal effects of mobile phone use.

It was reported that free radical mediated LPO is involved in EMR-induced tissue injury. First, microwave cooking was shown to increase MDA concentrations in fat from meat (35). However, our results show that there is an oxidative stress-induced LPO in kidney after mobile phone exposure, which returns to normal condition during the course of melatonin treatment. Likewise, MDA and NAG levels may be an important marker showing the degree of renal changes and the success of the treatment of renal impairment with melatonin.

It is reported that low frequency pulsed DC EMR exposure in rats also resulted in the augmentation in levels of free radicals and decreased the serum levels of melatonin, which is an efficient free radical scavenger. This fall was explained by an increased uptake of melatonin by tissues that were experiencing oxidant stress (36). In addition, treatment of rats before and after EMR exposure with melatonin (37) and vitamin E (38) was found to block the adverse effect of EMR in rat brain, possibly by affecting the lifetime of the radicals. Other arguments are provided to suggest that the encounters of freely diffusing pairs of radicals are unlikely to produce significant biological effects (39–41).

SOD, which is activated by melatonin, is a specific antioxidant enzyme dismutate O₂⁻ forming H₂O₂, which is scavenged by peroxisomal CAT or GSH-Px. Three SODs, copper/zinc SOD (cytosolic SOD), manganese SOD (mitochondrial SOD), and extracellular SOD (ECSOD), are major antioxidant enzymes based on cellular distribution and localization. Of the three isoforms, EC-SOD may be the most important in blood vessels, accounting for up to 70% of the total activity of SOD in blood vessels (42).

The distribution of extracellular SOD in the vessel wall of renal tissue seems ideal for detoxifying superoxide anions produced in sera of EMR-exposed rats. The extremely rapid interconversion of ROS (O_2^- , H_2O_2) within the cell can make it difficult to identify the originating species. The mitochondrial respiratory chain is the major site for the generation of superoxide radicals (O_2^-) (43). It is possible that EMR may affect the mitochondrial membranes to produce large amounts of oxygen radicals. Cyclooxygenase-2 (COX-2) activation and neutrophil infiltration may also be other sources of ROS under our experimental conditions (44,45).

These continuously produced ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (CAT). Under some circumstances, these endogenous antioxidative defenses are likely to be perturbed as a result of overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue. It has been demonstrated in numerous studies that ROS are directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues.

Administration of melatonin significantly stimulated the reduced activities of SOD, GSH-Px and CAT in kidney tissue over those measured in the EMR-exposed and control groups. In addition to the directly detoxifying property of melatonin, melatonin stimulates gene expression and activities of SOD which dismutates O_2^- to H_2O_2 (46). Thereafter, the two enzymes assigned the function of metabolizing H_2O_2 , i.e., CAT and GSH-Px, and thereby reducing the formation of highly toxic OH, have been shown to be stimulated by melatonin as well (47–49). These indirect antioxidative actions of melatonin might certainly magnify its protective actions in the kidney tissue under conditions of high oxidative stress. Our results are in agreement with previous data (46–50).

Ameliorating effects of melatonin in renal failure-induced oxidative damage were reported (51). According to this study, the increase in MDA and NAG and decrease of glutathione levels in different tissues, reflecting of oxidant-induced tissue damage, were reversed with melatonin treatment in concordance with the present study. Likewise, the reduced enzymatic antioxidant activity as a result of oxidative stress in kidney was also restored with melatonin. These effects were attributed to the stimulatory role of melatonin on antioxidant enzymes.

Our results may indicate a probable role of ROS in the adverse effects of EMR from a cellular telephone and also indicate that melatonin prevents these effects. However, we also know that it is difficult to extrapolate effects from rodents to humans because the entire body of a rat is exposed whereas for a person using a mobile telephone, only the skin near the region of the kidney that is close to the telephone would be exposed.

Thus, it may also be said that this pathological process could be prevented by an efficient scavenger. Herein, there

is a question: which agent should be selected? The answer may be melatonin, according to our current data.

In conclusion, in view of previous observations and our data, the potent free radical scavenger and antioxidant agent, melatonin, seems to be a highly promising agent for protecting renal tissue from oxidative damage and preventing organ dysfunction as a result of 900-MHz exposure. However, there is a need for further studies with different frequencies and exposure periods in order to prove the protective effect of melatonin on EMR-induced oxidative stress in kidney.

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