

Oxidative stress in hippocampus induced by 900 MHz electromagnetic field emitting mobile phone: Protection by melatonin

Memduh Kerman¹, Nilgun Senol²

¹Department of Neurosurgery, Sema Hospital, Istanbul, Turkey

²Department of Neurosurgery, Suleyman Demirel University School of Medicine, Isparta, Turkey

Abstract

The mobile phones emitting electromagnetic radiation (EMR) may be mainly absorbed by brain tissue since they are often used nearly skull. 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) in neural tissue damage in hippocampus and the role of melatonin against possible oxidative neural tissue damage in hippocampus in rats. Animals were randomly grouped as follows: 1) Cage control group 2) Control group 3) Study groups: i) 900 MHz EMR exposed (30 min/day for 10 days) group and ii) 900 MHz EMR exposed + melatonin (100 µg kg⁻¹ s.c. before the daily EMR exposure) treated group. Malondialdehyde (MDA, an index of lipid peroxidation), was used as a marker of oxidative stress-induced neural tissue impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate the changes of antioxidant status. In the EMR exposed group, while tissue MDA levels increased, SOD, CAT, and GSH-Px activities were reduced. Melatonin treatment reversed these effects as well. In this study, increase in MDA levels and decrease in SOD, CAT, and 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 hippocampus. These results show that melatonin may exhibit a protective effect on mobile phone-induced neural tissue impairment in hippocampus in rats.

Key words: melatonin, hippocampus, 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 [2,3,4]. The use of cellular phones in last years has raised many questions about their use of is safe, 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 presented that different system diseases are related to EMR such as mobile phones [6,7,8,9].

Mobile phones-induced free radical formation in other tissues has been reported [10,11,12]. Biological systems may interact resonantly with EMR 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 (O₂⁻), which is predominantly generated by the mitochondria; hydrogen peroxide (H₂O₂) produced from O₂ by the action of superoxide dismutase (SOD), and peroxynitrite (ONOO⁻), generated by the reaction of O₂ with nitric oxide (NO). ROS are scavenged by SOD, glutathione peroxidase (GSH-Px) and catalase (CAT).

The brain is a major potential route for the absorption of hazardous materials encountered in the environmental place [10]. The mobile telephone emitted 900 MHz radiation may absorbed by neural tissue more than other organs. 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 neural tissue and other organs [12]. Levels of

these endogenous indices of oxidative stress have not yet been reported in EMR-exposed animals.

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 H₂O [13]. The marked protective effects of melatonin against oxidative stress are aided by its ability to cross all biological membranes [13,14,15].

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

Materials and methods

Animal Model

Male Wistar-Albino rats (8 weeks old, 150 g) obtained from Laboratory Animal Production Unit of Suleyman Demirel University was used in the study. They were kept in an environment of controlled temperature (24–26°C), humidity (55–60%), and controlled photoperiod (12 h of light and 12 h of dark) for one week before the start of 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) A 900 MHz EMR exposed (30 min/day for 10 days) group (n = 8) and ii) A 900 MHz EMR exposed + melatonin treated group (n = 8). Melatonin (Sigma Chemical Co., USA) was injected s.c. daily at 17:00 hr a dose of 100 µgkg⁻¹ with dissolved in 1:90 ethanol/saline.

Before EMR exposure, animals were anaesthetized with pentobarbital Na, 40-60 mg/kg. A 900 MHz EMR was applied to study groups for 10 days (1/2 h/day, at 5:p.m. daily). Sham-operated rats (Group I) received isotonic saline solution (an equal volume of melatonin) was administered and were also anaesthetized 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 study, rats were sacrificed, and the brains dissected out. One gram of brain tissue was homogenized in a motor-driven tissue homogenizer with phosphate buffer (pH 7.4). Unbroken cells, cell debris and nuclei were sedimented at 2,000 g for 10 min, and the supernatant was pipetted into plastic tubes, and stored at -70°C until assayed.

Exposure device

900 MHz continuous wave electromagnetic energy generator (2 W peak powers, average power density 1,04 mW/cm²) was used in the study. The power density measurements were made using electromagnetic field meter (Holaday Industry Inc.), 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 the 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 [16]. 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 co-efficient = 1.56 × 10⁵ cm⁻¹ M⁻¹) and is expressed as nano moles per gram units (nM g⁻¹) wet tissue.

Determination of SOD Activity

Total (Cu-Zn and Mn) SOD activity was determined according to the method of Sun et al. [17] with a slight modification by Durak et al. [18]. 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 [19]. The enzymatic reaction in the tube that contained reduced nicoti-

namide 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 [20]. The principle of the assay is based on the determination of the rate constant k (dimension: s^{-1} , k) of H_2O_2 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.

Tissue protein was determined with Lowry method [21].

Statistical Analysis

Data was presented as means \pm SD. A computer program (SPSS 9.0, SPSS Inc. Chicago, IL, USA) was used for statistical analysis. A one way ANOVA test was applied to data to detect significant differences initially. At the second step, the 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, exposure to mobile phone device produced a significant decrease in activities of antioxidant enzymes (CAT, SOD and GSH-Px) in hippocampal neural tissue while tissue levels of MDA EMR exposed group were higher than those of the control group (Table 1, Figure 1). However, melatonin administration increased activities of antioxidant enzymes (show antioxidant status) and significantly reduced MDA levels (shows LPO degree) compared with the EMR group ($P < 0.001$, Table 1, Fig 2). The results are summarized in Table 1 and Figure 1.

Discussion

The close proximity of the antenna of such a device - the mobile phone - to the body has raised concerns about the biological interactions between EMR and the hippocampal tissue. The direct biological effects of exposure to 900 MHz EMR in the brain tissue have not been studied extensively.

The present study has shown that exposure to EMR with a frequency of 900 MHz has significant effect on rat hippocampus, suggesting that oxygen free radicals were generated under the experimental conditions employed. But, we observed a significant decrease in serum SOD activity in

the exposed group, and SOD activity was positively associated with GSH-Px activity. These results suggest that EMR induces an oxidative stress within the brain tissue of rats. The change in SOD activity may be regarded as an indicator of increased ROS production occurring during the exposure period and may reflect the pathophysiological process of the exposure.

There are several reports which indicate that free radicals are involved in EMR induced tissue injury [10,11,12]. First, microwave cooking was shown to increase MDA concentrations in fat from meat 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 [22]. This fall was explained by an increased uptake of melatonin by tissues that were experiencing oxidant stress [23]. In addition, treatment of rats before and after EMR exposure with melatonin [24] and vitamin E [25] was found to block the adverse effect of EMR, possibly by affecting the lifetime of the radicals. Indication of oxidative stress observed in brain seems paradoxical since neuronal cells are well known to have a higher rate of oxidative metabolic activity, and possess higher concentrations of readily oxidizable membrane polyunsaturated fatty acids than other organs [26]. However, the skull seems not to protect this sensitive organ from adverse effects of EMR.

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].

Localizations of specific melatonin receptors (ML-1 receptor) have been found in neural tissue [28,29]. This finding is highly suggestive of a direct neural functional role for melatonin. Thus, the presence in neural tissue of specific

ML-1 receptor has strong biological implications, suggesting that the cellular action of melatonin is effected through membrane transduction and intracellular signaling following melatonin binding to its receptor.

It was observed that a significant increase in MDA levels in the exposed group. The change in activities of antioxidant enzymes with MDA may be regarded as an indicator of increased ROS production occurring during the exposure period and may reflect the pathophysiological process of the exposure. However; our results show that there is an oxidative stress-induced LPO in after mobile phone exposure which comes back to normal condition during the course of melatonin treatment. Likewise, MDA levels may be an important marker showing the degree of neural

changes and the success of the treatment of neural impairment with melatonin.

These continuously-produced ROS are scavenged by SOD, GSH-Px and 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 group. Besides the directly detoxifying property of melatonin, melatonin stimulates gene expression and activities of SOD which dismutates O_2^- to H_2O_2 [29].

Thereafter, the two enzymes assigned the function of metabolizing H_2O_2 , i.e. CAT and GSH-Px, and thereby reducing the formation highly toxic $\cdot OH$, have been shown to be stimulated by melatonin as well [13,30]. These indirectly anti-oxidative actions of melatonin might certainly magnify its protective actions in the neural tissue under conditions of high oxidative stress. Our results are in accordance with this previous data [31,32,33].

The reduced enzymatic antioxidant activity as a result of oxidative stress in the brain was also restored with melatonin. These data was attributed to 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 prevented 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 throughout skull to brain tissue that is close to the telephone would be exposed.

Thus, it may also be said that this pathological process should be prevent 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 the 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 hippocampal neural tissue.

References

1. Repacholi MH. Health risks from the use of mobile phones. *Toxicology Letters* 2001; 120(1-3): 323-31.
2. Rothman KJ, Loughlin JE, Funch DP, Dreyer NA. Overall mortality of cellular telephone customers. *Epidemiology* 1996; 7: 303-305.
3. Somosy Z. Radiation response of cell organelles. *Micron* 2000; 31: 165-181.
4. Cox DR. Communication of risk: health hazards from mobile phones. *Journal of the Royal Statistical Society: Series A (Statistics in Society)* 2003;166: 241-45.
5. Knave B. Electromagnetic fields and health outcomes. *Annals of Academy of Medicine Singapore* 2001; 30 (5): 489-493.
6. Leszczynski D, Joenvaara S, Reivinen J, Kuokka R. Non-thermal activation of the sp27/p38MAPK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and blood-brain barrier-related effects. *Differentiation* 2002; 70: 120-129.
7. Bartsch H, Bartsch C, Seebald E, Deerberg F, Dietz K, Vollrath L, Mecke D. Chronic exposure to a GSM-like signal (mobile phone) does not stimulate the development of DMBA-induced mammary tumors in rats: results of three consecutive studies. *Radiation Research* 2002; 157 (2): 183-190.
8. Bortkiewicz A. A study on the biological effects of exposure mobile-phone frequency EMF. *Medical Practice* 2001; 52 (2): 101-106.
9. Riu PJ, Foster KR, Blick DW, Adair ER. A thermal model for human thresholds of microwave-evoked warmth sensations. *Bioelectromagnetics* 1997; 18 (8): 578-583.
10. Irmak MK, Fadillioglu E, Gulec M, Erdogan H, Yagmurca M, Akyol O. Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. *Cell Biochemistry and Function* 2002; 20: 279-283.
11. Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M, Akyol O, Ozen S. Ginkgo biloba prevents mobile phone-induced oxidative stress in rat brain. *Clinica Chimica Acta* 2004; 340: 153-162.
12. Irmak MK, Oztas E, Yagmurca M, Fadillioglu E, Bakir B. Effects of electromagnetic radiation from a cellular telephone on epidermal Merkel cells. *Journal of Cutaneous Pathology* 2003; 30: 135-138.
13. Tan DX, Poeggeler B, Reiter RJ, Chen LD, Chen S, Manchester LC, Barlow WLR. The pineal hormone melatonin inhibits DNA-adduct formation induced by the chemical carcinogen safrol in vivo. *Cancer Letters* 1993; 70: 65-71.
14. Reiter RJ, Tan DX, Osuna C. Actions of melatonin in the reduction of oxidative stress. *Journal of Biomedical Science* 2000; 7: 444-458.

15. Cuzzocrea S, Reiter RJ. Pharmacological action of melatonin in shock, inflammation and ischemia/reperfusion injury. *European Journal of Pharmacology* 2001; 426: 1-10.
16. Draper HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology* 1990; 186: 421-431.
17. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. *Clinical Chemistry* 1988; 34: 497-500.
18. Durak I, Yurtarlani Z, Canbolat O, Akyol O. A methodological approach to superoxide dismutase (SOD) activity assay based on inhibition of nitroblue tetrazolium (NBT) reduction. *Clinica Chimica Acta* 1993;214: 103-04.
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *Journal of Laboratory and Clinical Medicine* 1967; 70: 158-169.
20. Aebi H. Catalase In: Bergmeyer HU, editor. *Methods of enzymatic analysis*. New York and London: Academic Press 1974; 673-677.
21. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. *Clinical Chemistry* 1951; 193: 265-269.
22. Abdel-Kader ZM. Lipid oxidation in chicken as affected by cooking and frozen storage. *Nahrung- Food* 1996; 40: 21-24.
23. Reiter RJ, Tan DX, Poeggeler B, Kavet R. Inconsistent suppression of nocturnal pineal melatonin synthesis and serum melatonin levels in rats exposed to pulsed DC magnetic fields. *Bioelectromagnetics* 1998; 19: 318-329.
24. Lai H, Singh NP. Melatonin and a spin-trap compound block radiofrequency electromagnetic radiation-induced DNA strand breaks in rat brain cells. *Bioelectromagnetics* 1997; 18: 446-454.
25. Koana T, Okada MO, Ikehata M, Nakagawa M. Increase in the mitotic recombination frequency in *Drosophila melanogaster* by magnetic field exposure and its suppression by vitamin E supplement. *Mutation Research* 1997; 373: 55-60.
26. Evans PH. Free radicals in brain metabolism and pathology. *British Medical Bulletin* 1993;49: 577-87.
27. Braune S, Wrocklage C, Raczek J, Galius T, Lucking CH. Resting blood pressure increase during exposure to a radiofrequency electromagnetic field. *Lancet* 1998; 351: 1857-1858.
28. Masson-Pevet M, George D, Kalsbeek A, Sabourau M, Lakhdar-Ghazal N, Pevet P. An attempt to correlate brain areas containing melatonin-binding sites with rhythmic function: a study in five hibernator species. *Cell and Tissue Research* 1994; 278: 97-106.
29. Gauer F, Masson-Pevet M, Pevet P. Seasonal regulation of melatonin receptors in rodent pars tuberalis: correlation with reproductive state. *Journal of Neural Transmission* 1994; 96: 187-195.
30. Tan DX, Manchester LJ, Reiter R. Melatonin directly scavenges hydrogen peroxide: a potentially new pathway of melatonin biotransformation. *Free Radical Biology & Medicine* 2000; 29: 1177-1185.
31. Antolin I, Rodriguez C, Sainz RM. Neurohormone melatonin prevents cell damage. Effect on gene expression for antioxidant enzymes. *The Federation of American Societies for Experimental Biology* 1996; 10: 882-890.
32. Okatani Y, Wakatsiku A, Shinohara K, Kaneda C, Fukaya T. Melatonin stimulates Glutathione peroxidase activity in human chorion. *Journal of Pineal Research* 2001; 30: 199-205.
33. Liu F, Ng TB. Effect of pineal indoles of the antioxidant enzymes superoxide dismutase, catalase glutathione reductase, and levels of reduced and oxidized glutathione in rat tissues. *Biochemistry and Cell Biology* 2000; 78: 447-453.

Correspondence to:

Memduh Kerman
Sema Hastanesi
Istanbul, TURKEY
Phone : 00 90 216 4589054
Fax : 00 90 216 4570331
E-mail : memduhkerman@yahoo.com