Health Risks of Electromagnetic Radiation from Mobile Phone on Brain of Rats

Sahar M. Awad and Nahed S. Hassan

Department of Physics, Faculty of Science, Alazhar University, Cairo Egypt.
Department of Biochemistry, National Research Center, Cairo Egypt.

Abstract: Tremendous concerns have been raised about the possibility that exposure to the electromagnetic radiation (EMR) from mobile phones could affect people’s health. This study was carried out in order to investigate the impact of exposure to the (EMR) of mobile phones. Since recent experimental studies suggest a possible link between mobile phone use and reactive oxygen species (ROS) in EMR-induced oxidative damage in tissues. In this study, rats were divided into three groups, The first group was used as control group and the other Two groups were exposed to 900 MHz EMR from mobile phone for one week (1 h/day) and for two weeks (1 h/day). Control group was prepared by turning off the mobile phone while the animals were in the same exposure conditions (sham exposed to EMR). Subsequently, oxidative stress markers and pathological changes in brain tissue were examined for all groups. The results indicated significant increase in plasma lipid peroxide (PLPO) and malondialdehyde (MDA) levels. Also there was significant decrease in brain superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and glutathione peroxidase (GSH-Px) activities in brain tissue. These alterations were indicative for oxidative damage and disturbance in antioxidant system. Histopathological studies revealed cellular injury in brain tissue induced by mobile phone EMR exposure. In conclusion reactive oxygen species may play a role in the mechanism that has been proposed to explain the biological side effects of mobile phone (MP) in brain tissue.

Key words: Electromagnetic radiation, Mobile phones, Brain, Rat

INTRODUCTION

Concern continues about exposure to radiofrequency (RF) fields which are increasingly available in our daily environment, for example, mobile phone (MP) transmitters/ receivers, radars, satellites, radio/TV transmitters, video display terminals, microwave ovens and occupational devices[1,2]. A particular concern has been raised about the possibility that exposure to the radiofrequency fields emitted by MPs could affect people’s health and recent alarming reports demand further investigations on this subjects[3]. MPs operate on wireless technology, with communication typically occurring via a 900-1800 MHz signal that is pulsed at 217 Hz. This signal carries essentially no power when the user is not talking or receiving, but when the user communicates the power of this pulsed electromagnetic field reaches a maximum of 250 mW[4].

The duration period for mobile phone use and its frequency are important factors, but the exact duration differs from individual to individual. 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[5,6]. 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 ocular symptoms[7,8].

However, mobile phone antennas give localized RF exposures predominantly to the head. Thus, it is necessary to determine the local SAR and its distribution in the head to properly evaluate health consequences. Calculation of the maximum temperature rise in the head from RF exposure during mobile telephone use suggests that increases of no more than about 0.1°C would be expected[9,10]. Thus if there are health effects from RF exposure, they are unlikely to be due to any temperature increase. So-called non-thermal mechanisms of RF action in tissues have been proposed[1,2].

There are many studies in the literature about the biological interactions with EMF and the direct biological effects which such exposure could originate[11-13]. Recent in vitro and in vivo studies observed the occurrence of DNA damage[14-16], as well as micronucleus (MN) generation, which is a well-accepted index for genotoxicity evaluation, after the EMF exposure[17-19]. However, it is not clear how EMF interacts with living systems. Some authors pointed out a possible role of oxidative stress in this process, and proposed mathematical models explaining how weak
electromagnetic fields could impair radical recombination, thus increasing free radicals generation[28,29]. In agreement with this hypothesis some authors suggested that EMF might also increase free radicals formation, based on the assumption that ROS are implicated in several types of tissue injury[22-26]. ROS are scavenged by SOD, and also the enzymes glutathione peroxidase (GSH-Px) and catalase (CAT)[27,28]. The aim of the current study is to investigate the possible harmful effects of exposure to brain tissue to emitting levels of EMR from mobile phones in the rats, focusing on changes in the antioxidant enzyme activities and various oxidant parameters of the brain.

MATERIALS AND METHODS

Animal model: 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 National Research Center, Animal Ethical Committee. Twenty four Male Wistar Albino rats obtained from the Laboratory Animal Production Unit of National Research Center were used in the study, (each weighing 250-300 g and approximately 3 months old at the time of the experiment). They were housed individually in polycarbonate cages. The housing room was kept in an environment of controlled temperature (24-26 °C), humidity (55-60%), and controlled photoperiod (12h/12h of light and dark) for 1 week before the start of the experiment. A standard balanced diet and tap water were provided ad libitum.

The exposure system: consisted of a plastic tube cage (length: 15 cm, diameter: 6.5 cm) and a mobile phone antenna. Unanesthetized male Wistar rats were confined in the cages. The heads of the rats were positioned toward the antennas of the mobile phones, and each tube was ventilated to decrease the stress of the rat while in the tube. This exposure system was based on a previous report of Tsurita et al.[28]. In the present study, a 900 MHz electromagnetic near-field signal for GSM (Global System for Mobile communication at 900 MHz, continuous wave, analog phone) system was used. The peak specific absorption rate (SAR) of the brain was 2 W/kg and the average SAR of the whole body was 0.25 W/kg.

Experimental design: Animals were randomly grouped as follows: group I (n=8), sham-operated control group, group II (n=8) rats were exposed to EMR in the above referred conditions for 1 h/day for 7 days and group III (n=8) rats were exposed to EMR for 1 h/day for 15 days. Mobile phones were activated by calling each other. The rats of control group were also placed in the tube with the same environmental room conditions as the exposure groups but without exposure to EMR (mobile phone off).

Biochemical evaluation: To evaluate the biological effects of the EMR exposure from mobile phone to the brain, the antioxidant enzyme activity of the brain tissue was evaluated. Brains were removed immediately after decapitation and sectioned sagittally as right and left hemispheres. Right hemispheres were washed twice with cold saline solution, placed into glass bottles, labeled and stored at 30 °C until processing (maximum 10 h). Tissues were homogenized in a four volumes of ice-cold Tris - HCl buffer (50 mmol/l, pH 7.4) using a glass Teflon homogenizer (Ultra Turrax IKA T18 Basic) after cutting of the brains into small pieces with a scissors (for 2 min at 5000 rpm) Malondialdehyde (MDA) activity was carried out at this stage. The homogenate was then centrifuged at 5000 × g for 60 min to remove debris. Clear upper supernatant fluid was taken, glutathione peroxidase (GSH-Px), Glutathione Reductase (GR) and catalase (CAT) activities were carried out in this stage. The supernatant solution was extracted with an equal volume of an ethanol/ chloroform mixture (5/3, volume per volume [v/v]). After centrifugation at 5000 × g for 30 min, the clear upper layer (the ethanol phase) was taken and used in the superoxide dismutase (SOD) activity.

All preparation procedures were performed at 4 °C.

Determination of MDA: MDA levels were estimated by the double heating method of Draper and Hadley[29]. The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA and is expressed as nanomoles/gram (nM g⁻¹) wet tissue.

Determination of SOD activity: Total (Cu-Zn and Mn) SOD activity was determined according to the method of Durak et al.[30]. 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 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[31]. 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 (H₂O₂) 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: Catalase (CAT) activity was measured according to the method of Aebi[32]. 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
Determination of GR activity: Glutathione reductase (GR) was assayed by the method of Stall et al.\cite{33} and expressed as nmol g⁻¹ protein.

Determination of PLPO activity: Blood was collected from the rats in all previous mentioned groups. Plasma was separated and kept in 80 °C until analyzed for plasma lipid peroxide activity (PLPO) using the method of Yagi\cite{34} and expressed as nmol ml⁻¹ s⁻¹.

Histopathological examination: After removing the brain from skull, brains were sectioned sagittally. Right hemispheres were removed and fixed with a buffered 10% formalin solution for 24 h and embedded in paraffin. Tissues were then sectioned at 5 Am, stained with hematoxylin and eosin (H&E) and examined for histopathological changes using light microscope. The occurrence of ‘‘dark neurons’’ was judged semi-quantitatively by the pathologist as A (non occasional dark neurons), B (moderate occurrence of dark neurons) and C (abundant occurrence of dark neurons).

Statistical analysis: Data were presented as means ± SE. All analyses were made using the SPSS statistical software package. 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 AND DISCUSSION

Results: To estimate the role of reactive oxygen species induced by exposure to EMR from mobile phone for one and two weeks, LPO degree and antioxidant status were determined in sham exposed (control) and EMR-exposed groups. Exposure to electromagnetic radiation ‘‘EMR’’ from mobile phone device produced a significant increase in tissue level of MDA and plasma lipid peroxides (indicator of LPO degree) in group II (EMR-exposed for one week, p <0.05) and group III (EMR- exposed for two weeks, p <0.01) as compared with control sham exposed group I, (Table I, Figure1). While activities of antioxidant enzymes (indicator of antioxidant status) were found to be decreased in EMR-exposed groups when compared with control sham exposed group I (Table I, Figure 1).

The level of SOD activity was decreased in group II (p < 0.01) and group III (p < 0.001) and values of GSH-Px activity was found to be decreased in EMR group (p < 0.05). Also EMR exposure revealed a non significant decrease in CAT activity in group II and significant decrease in group III (p < 0.01). EMR induced significant decrease in GR activity in both group II (p < 0.05) and group III (p < 0.001) when compared to those of control

Values as shown in (Table 2, Figure 2).

The pathological examination revealed a significant positive relation between EMR exposure duration time and number of dark neurons appeared as scattered and grouped neuron cells, which were often interspersed, shrunken nerve cells and dark staining so called dark neurons.

Dark neurons were seen in all locations, but especially in the cortex, hippocampus and basal ganglia, mixed in among normal neurons (Figure3). The number of dark neurons increased as the duration time of EMR exposure increased in group II and III, on comparing with the sham exposed group I.

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 head and consequently to the brain has raised concerns about the biological interactions between EMR and the brain tissues. The direct biological effects of exposure to 900-MHz EMR have not been studied extensively.

The present study has shown that exposure to EMR with a frequency of 900 MHz had a significant effect on rat brain, suggesting that ROS were generated under the experimental conditions employed. A significant increase was observed in MDA and PLPO levels in the exposed group. The change in activities of antioxidant enzymes with MDA and PLPO 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. Thus, impaired oxidant/antioxidant balance in brain might be partially responsible for the adverse effects of mobile phone use. It was reported that free radical mediated LPO is involved in EMR-induced tissue injury\cite{35}. Moustafa et al\cite{36} indicated that acute exposure to EMR may modulate the oxidative stress of free radicals by enhancing lipid peroxidation and reducing the activation of SOD and GSH-Px, which are free radical scavengers. Irmak et al\cite{36} have shown that EMR with frequency of 900 MHz has no significant effect on rabbit brain, suggesting that oxygen free radicals were not generated; but, they observed a significant increase in serum SOD activity in the exposed group.

However, our results have revealed that there is an oxidative stress-induced LPO in brain after mobile phone exposure. Likewise, MDA and PLPO levels may be an important marker showing the degree of impairment in oxidant/antioxidant balance in brain. Comparative evaluation of our study with previous studies mentioned above has revealed an agreement in results obtained.
Table 1: Mean values of plasma lipid peroxide activity and level of MDA in brain tissues of rats.

<table>
<thead>
<tr>
<th>Parameters of LPO Degree</th>
<th>Plasma Lipid Peroxide (nmol ml⁻¹)</th>
<th>MDA (nM g⁻¹ wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Exposed Control Group I, (n=8)</td>
<td>3.002 ± 0.32</td>
<td>12.31 ± 2.19</td>
</tr>
<tr>
<td>EMR-Exposed for one Week Group II, (n=8)</td>
<td>3.881 ± 0.37</td>
<td>18.16 ± 1.82</td>
</tr>
<tr>
<td>EMR-Exposed for two Weeks Group II, (n=8)</td>
<td>4.011 ± 0.26</td>
<td>19.62 ± 2.13</td>
</tr>
</tbody>
</table>

P Value

| I — II | P < 0.05 | P < 0.05 |
| I — II I | P < 0.01 | P < 0.01 |
| II — III | NS | NS |

Fig. 1: Levels of plasma lipid peroxide and MDA in brain tissues of rats in shame exposed control, EMR-exposed for one week and EMR-exposed for two weeks groups.

Table 2: Mean values of antioxidant enzymes activities in brain tissues of rats.

<table>
<thead>
<tr>
<th>Parameters of Antioxidant Status</th>
<th>SOD (U g⁻¹ protein)</th>
<th>GSH-PX (U g⁻¹ protein)</th>
<th>CAT (K g⁻¹ protein)</th>
<th>GR (nM g⁻¹ protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham Exposed Control Group I, (n=8)</td>
<td>0.64±0.041</td>
<td>1.861±0.231</td>
<td>4.06 ±0.21</td>
<td>1.29±0.098</td>
</tr>
<tr>
<td>EMR-Exposed for one Week Group II, (n=8)</td>
<td>0.49±0.037</td>
<td>1.326±0.162</td>
<td>3.67±0.19</td>
<td>0.96±0.121</td>
</tr>
<tr>
<td>EMR-Exposed for two Weeks Group II, (n=8)</td>
<td>0.41±0.052</td>
<td>1.21±0.201</td>
<td>3.12±0.24</td>
<td>0.72±0.113</td>
</tr>
</tbody>
</table>

P Value

| I — II | P < 0.05 | P < 0.05 |
| I — II I | P < 0.01 | P < 0.01 |
| II — III | NS | NS |

Kula et al.[37] demonstrated that the degree of oxidative damage on the biomolecular level can be determined via the assessment of antioxidant enzyme activities and the densities of mid- and end products of lipid peroxidation. MDA exists among the end products of lipid peroxidation. At the end of the reaction, MDA is produced alongside many compounds such as alkenes, 2-alkenals, and 4-hydroxyalkenals. An increase of MDA level indicates the severity of oxidative damage. In this study, MDA levels increased on days 7 and 14 on comparison with controls, It has been reported by various researchers that exposure to EMF of 50 Hz and at different magnitudes caused lipid peroxidation in different living species[38,39].

It was documented by Albers and Beal[40] that the mitochondrial respiratory chain is the major site for the generation of superoxide radicals (O²⁻, H₂O₂). It is possible that EMR may affect the mitochondrial membranes to produce large amounts of oxygen radicals ROS under our experimental conditions. These
Fig. 2: Activities of antioxidant enzymes in brain tissues of rats in sham exposed control, EMR-exposed for one week and EMR-exposed for two weeks groups.

Fig. 3: Histopathological appearance of hippocampus in brain tissues of rats in (A) sham exposed control, (B) EMR-exposed for one week and (C) EMR-exposed for two weeks groups.

continuously produced ROS are scavenged by SOD, glutathione peroxidase (GSH-Px), catalase (CAT) and glutathione reductase (GR). 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.

Salford et al., first reported the evidence for neuronal damage caused by nonthermal microwave exposure. The cortex as well as the hippocampus and the basal ganglia in the brains of exposed rats contain dark neurons. Ilhan et al., demonstrated that mobile phones caused oxidative damage biochemically and produced histopathological changes in brain tissue in a rat model exposed to EMR. They suggested that there was significant relationship between EMR dose and number of dark neurons, which have been causally linked to the generation of ROS and oxidative stress. This was concomitant with results observed in this study (Figure 3).

Scientists have warned children for the possible hazardous effect of mobile phones, since young persons are growing up. Indeed, in young individuals, cellular defenses against free radical-induced protein oxidation by antioxidant enzymes are likely in prime shape and the proteases and protein synthesis machinery are fully functional. Thus, there is little or no change in the level of protein carboxyls during the first 45 years. However, when normal individuals reach 45 years, enzymes, including proteases and antioxidant proteins, and smaller antioxidant molecules in the individual become progressively inactivated due to the failure of the antioxidant systems to overcome the constant influx of ROS. Consequently, the accumulation of free radical-induced carboxylated proteins accelerates, indicating the age when cells in the individual become increasingly more susceptible to ROS-mediated damage. Since cumulated oxidative stress in brain cells can lead to neurodegenerative diseases and an excess of free radicals in cells has been suggested to be the cause of various human diseases (Parkinson’s disease, Alzheimer’s disease and amyotrophic lateral sclerosis, etc.). On the basis of Ilhan et al., findings elder people must be more cautious for EMR-induced
Oxidative damage. Although present scientific information does not indicate the need for any special precautions for use of mobile phones, if individuals are concerned, they might choose to limit their own EMR exposure by limiting the length of calls, or using "hands-free" devices to keep mobile phones away from the head and body.

REFERENCES


