

The influence of microwave radiation from cellular phone on fetal rat brain

Ji Jing¹, Zhang Yuhua², Yang Xiao-qian³, Jiang Rongping⁴,
Guo Dong-mei¹ & Cui Xi¹

¹Institute of Chemistry and Bacteria Detection, Department of Public Health, Shandong University, Shandong, China, ²Department of Radiology, Shandong Qianfoshan Hospital, Shandong, China, ³CDC of Jinan, Shandong, China, and ⁴Chemical Technology Academy of Shandong Province, Shandong, China

The increasing use of cellular phones in our society has brought focus on the potential detrimental effects to human health by microwave radiation. The aim of our study was to evaluate the intensity of oxidative stress and the level of neurotransmitters in the brains of fetal rats chronically exposed to cellular phones. The experiment was performed on pregnant rats exposed to different intensities of microwave radiation from cellular phones. Thirty-two pregnant rats were randomly divided into four groups: CG, GL, GM, and GH. CG accepted no microwave radiation, GL group radiated 10 min each time, GM group radiated 30 min, and GH group radiated 60 min. The 3 experimental groups were radiated 3 times a day from the first pregnant day for consecutively 20 days, and on the 21st day, the fetal rats were taken and then the contents of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), noradrenaline (NE), dopamine (DA), and 5-hydroxyindole acetic acid (5-HT) in the brain were assayed. Compared with CG, there were significant differences ($P < 0.05$) found in the contents of SOD, GSH-Px, and MDA in GM and GH; the contents of SOD and GSH-Px decreased and the content of MDA increased. The significant content differences of NE and DA were found in fetal rat brains in GL and GH groups, with the GL group increased and the GH group decreased. Through this study, we concluded that receiving a certain period of microwave radiation from cellular phones during pregnancy has certain harm on fetal rat brains.

Keywords Cellular phone, Microwave radiation, Fetal rat, Oxidative stress, Neurotransmitters, Brain tissues

INTRODUCTION

Microwave radiation is a type of non ionizing electromagnetic radiation in the environment, and is a potential threat to human health. Today, non ionizing radiation has increasingly been used in industry, commerce, medicine, and for private purpose, especially in cellular phones.

With the developing of modern communication technology, the cellular phone, as the high-tech communication product in today's information age, has entered people's work and all areas of life. Global system for cellular communications

Address correspondence to Cui Xi, Institute of Chemistry and Bacteria Detection, Department of Public Health, Shandong University, Jinan, Shandong, 250012 China; E-mail: cuixi@sdu.edu.cn

(GSM, 800–900 MHz) has become the world's largest mobile telecommunication system (Valberg et al., 2007). Microwaves, the electromagnetic waves ranging in frequency from 300 MHz–300 GHz, exist everywhere in our daily environment, for example, in a microwave oven, satellite, and radio/TV transmission, cellular-phone transmitter receiver, video display terminal, and so on (Michaelson, 1982). The microwaves from cellular phones almost contain all the bands of electromagnetic spectrum, which can produce extremely low-frequency electromagnetic fields (EMFs) (Repacholi and Greenebaum, 1999).

EMFs can influence neuronal functions, including regulation of synaptic plasticity, neurotransmitter release, neuronal survival, learning, and memory (Sakatani and Hirose, 2002; Manikonda et al., 2007; Salford et al., 2003). It also induces cell death and inhibits the differentiation of neural stem cells into neurons during embryonic development (De luliis et al., 2009). The epidemiological studies suggested that low-energy microwave emitted from a cellular phone might cause biological effects, such as DNA damage and changes on oxidative metabolism (Ferreira et al., 2006). Antioxidants can reduce the effects of free radicals formed in the body. As important health promoters, antioxidants are increasingly being recognized in conditions such as cardiovascular problems, treatments of many forms of cancer, and aging (Irmak et al., 2003).

In order to protect human's health from the microwave damage, the relevant radiation limits have been given by many countries. The current limited guidelines for microwave from cellular phone in U.S. and Europe are 1.6 W/kg and 2.0 W/kg, respectively. New lower limits should also be used for children and/or pregnant women.

Due to the proximity of cellular phone antenna to the user's ear and head, the brain is inevitably exposed to EMFs with a relatively high specific absorption ratio (SAR), so the potentially danger from EMFs has been a concern of more and more people, especially by pregnant women.

The epidemiological research and experimental studies on cellular phone EMFs effects have been discussed by scholars all over the world, but the reported results were not consistent (Savitz, 1993). Numerous epidemiological studies have demonstrated an association between various disorders, including childhood leukemia, neurological effects, and neurodegenerative diseases, brain cancer, and Alzheimer's disease, and either occupational or residential exposure to EMFs generated by cellular phones (Feychting and Ahlbom, 1993; Floderus et al., 1994; Hardell and Sage, 2008; Odaci et al., 2008; Paglialonga et al., 2007). But there were also many opposite studies performed and their results reported (Grafstrom et al., 2008; Wagner et al., 1998). Therefore, the question, whether the EMFs from cellular phones were harmful, still remains to be answered.

In this study, the pregnant rats were radiated by a cellular phone, and then the levels of superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), malondialdehyde (MDA), *norepinephrine* (NE), dopamine (DA), and 5-hydroxyindole acetic acid (5-HT) in brain tissue of fetus were detected to investigate the possible influence of EMFs from cellular phone on the offspring brain development.

MATERIALS AND METHODS

Animals

The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals prepared by Animal Ethical Committee, Shandong University.

(Animals were obtained from The Center of Laboratory Animal (Jinan, China)) Thirty-two pregnant Wistar rats weighing 256 ± 2.69 g (mean \pm SD) were used. In order to obtain the pregnant rats, two male rats were placed in a cage with a female, and the onset of pregnancy was determined by vaginal smear. The pregnant rats were housed in a room without near sources of EMFs, one in each cage. All animals had access to standard laboratory food and tap water ad libitum. The housing room was maintained at $21-24^{\circ}\text{C}$ with $42 \pm 5\%$ relative humidity and had an alternating cycle of 12 h light (8 am–8 pm) and 12 h darkness.

Exposure Design

The exposure process was conducted in an iron wire tube cage (length: 24 cm; diameter: 7.0 cm). The clapboard in experimental cages could be regulated to restrict the activity of pregnant rats. The pregnant rats were randomly designed to four groups with eight rats in each one. The animals were placed into experimental cages in the first day of pregnancy and exposed to the radiation from a cellular phone for 20 days. During the call, the antenna of the phone was maintained close to the ear of pregnant rats, and the regulation was made at all times to keep the distance between the antenna and pregnant rat's ear, completely imitating human's way of calling. The animals were exposed to cellular phone radiation 3 times a day, and each time 8 rats were exposed for 10 min (GL), 8 rats for 30 min (GM), and 8 rats for 60 min (GH), respectively. The rats in the control group were also placed in an experimental cage as rats in the other groups for the same time, but without exposure to EMFs (cellular phone off).

To evaluate the potentially detrimental effects of the electromagnetic radiation exposure from cellular phone on the fetus' brain, the antioxidant enzymes activity and the monoamine neurotransmitter content of the brain tissue was assayed. On the 21st day, pregnant rats were decapitated, and the fetal rats were removed from the uterus. From each pregnant rat, we randomly selected five fetal rats, weighing and removing the brain tissue for the detection of SOD, MDA, GSH-Px, and monoamine neurotransmitters.

Antioxidant Enzymes Evaluation

The kits of SOD, MDA, and GSH-Px were obtained from Nanjing Jiancheng Institute of Biological Engineering (Figure 1 and Table 1).

Determination of SOD Activity

Cu-Zn SOD activity was determined according to the introduction provided by Nanjing Jiancheng Institute of Biological Engineering. The principle of the method is based briefly on the SOD specificity inhibition to superoxide anion free radical which was generated by the xanthine and xanthine oxidation system. Activity was assessed by spectrophotometer under 550 nm. One unit of SOD was defined as the enzyme amount causing 50% inhibition rate in 1 mL reaction solution. Activity was expressed as units per milligram protein (U/mgprot).

Determination of GSH-Px Activity

Glutathione peroxidase (GSH-Px) activity was also measured according to the method provided by Nanjing Jiancheng Institute of Biological Engineering. The enzymatic reaction in the tube can lead to the decrease of reduced glutathione which was initiated by the addition of hydrogen peroxide (H_2O_2) and the change in absorbance at 412 nm was monitored by a spectrophotometer. Activity was also given in units per milligram protein (U/mgprot).

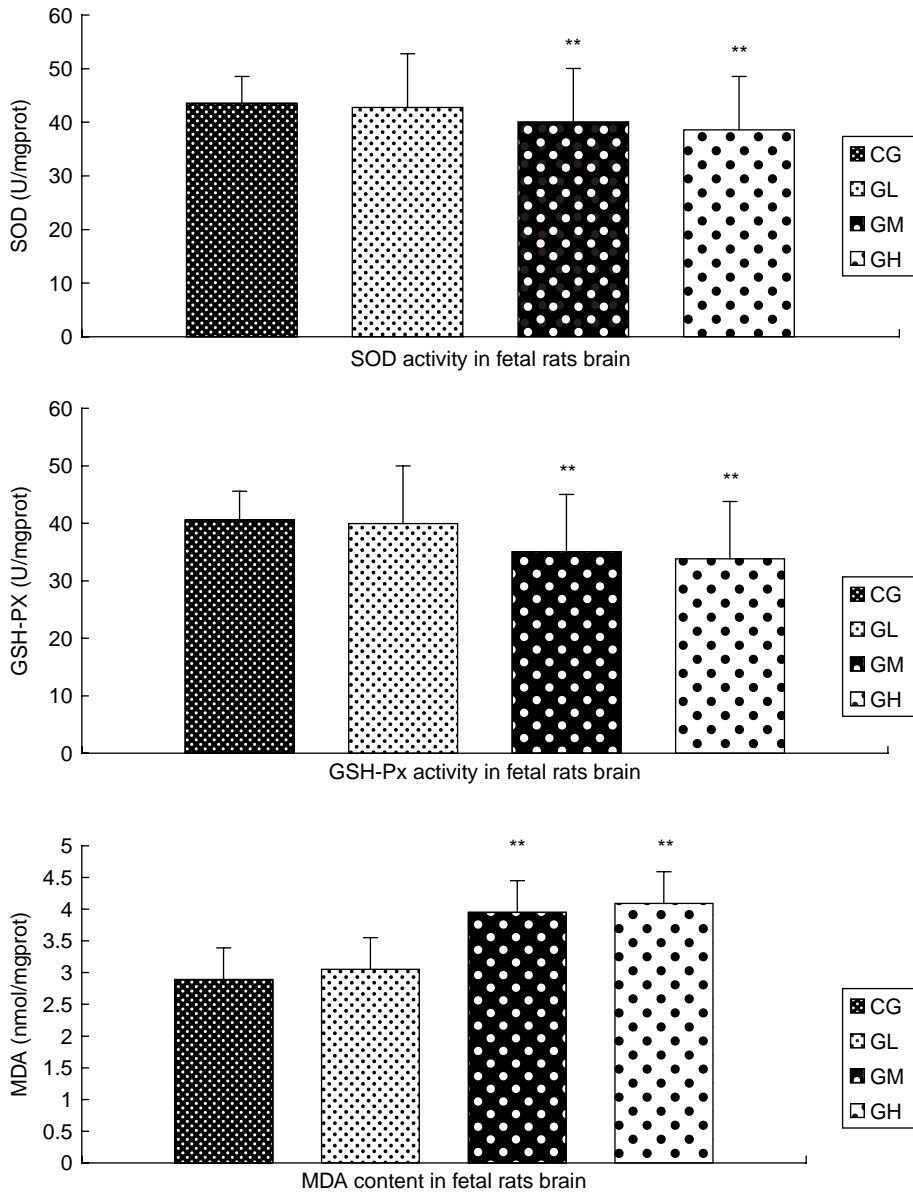


FIGURE 1 SOD, GSH-Px activity, and MDA content in brain tissues of fetal rats. ** $P < 0.05$ vs. control group.

TABLE 1 The activity of SOD, GSH-Px, MDA in fetal rats brain ($\bar{x} \pm s$).

Group	SOD (U/mgprot)	GSH-Px (U/mgprot)	MDA (nmol/mgprot)
CG	43.53 ± 5.38	40.58 ± 8.03	2.89 ± 0.57
GL	42.79 ± 5.06	39.95 ± 9.02	3.05 ± 0.76
GM	40.05 ± 4.74**	35.03 ± 7.18**	3.95 ± 0.64**
GH	38.56 ± 4.62**	33.80 ± 6.29**	4.09 ± 0.68**

**compared with CG, $P < 0.05$

Determination of MDA Activity

MDA level was estimated by the method provided by Nanjing Jiancheng Institute of Biological Engineering. The thiobarbituric acid (TBA) reacts with MDA, generating the color with the absorption at 532 nm. The concentration of MDA is expressed as nanomoles/milligram protein (nmol/mgprot).

Determination of Proteins

Brain protein was determined according to the reaction of Coomassie brilliant blue and protein standard. The content of protein was calculated by detecting the absorbance of the reaction solution.

Neurotransmitters Examination

The 5% perchloric acid was added to the brain tissue stored at -80°C with the proportion of 1 mL: 0.1 g (wet weight), and was homogenized under ice bath. The homogenate was collected in a centrifuge tube and centrifuged 20 min at 13,000 r/min. The supernatant was taken for the detection of NE, DA, 5-HT by high-performance liquid chromatography- fluorescence (HPLC-FL) detection.

Chromatographic Conditions

A Shimadzu 10Atp series HPLC system (Shimadzu Corporation, Nishinokyo Kuwabaracho, Kyoto, Japan) with FL detector was used. The FL detection wavelengths were set to $\lambda_{\text{ex}}/\lambda_{\text{em}} = 290/330$ nm. The chromatographic column was DIKMA C18 (250 mm \times 4.6 mm i.d., 10 μm). The mobile phase was methanol: water = 40:60 with the water containing 0.028 g/L EDTANa₂, 0.15 g/L SDS, 0.22 ml/L H₂SO₄ (pH 2.5–3). The flow-rate and column temperature were set at 1.0 ml/min and 35 $^{\circ}\text{C}$, respectively.

Statistical Analysis

Data were expressed as means \pm standard deviation (SD). Statistical analysis was carried out by variance (ANOVA) followed by appropriate post-hoc tests including multiple comparison tests (LSD). All analyses were made using the SPSS 13.0 statistical software package and $p < 0.05$ was considered significant.

RESULTS**The Activity of Antioxidant Enzymes**

Compared with the control group, the SOD and GSH-Px activities in brain tissue of GL group decreased with non significance. And the comparison of MDA contents in the brain tissue of above groups were also found to have no significance. SOD and GSH-Px activities decreased in GM and GH groups compared with those of control ($P < 0.05$). There was also significant difference in the content of MDA in GM and GH groups ($P < 0.05$).

The Content of Neurotransmitters

As shown in Table 2 and Fig. 2, compared with the control group, there were no significant differences found in the content of 5-HT among three groups. There were also no significant content differences of NE, DA in the GM group when compared with control group. The contents of NE, DA in the GL group increased significantly compared with those of control. The contents of NE, DA in the GH group decreased significantly when compared with those of control.

TABLE 2 The content of neurotransmitters in fetal rats brain ($\bar{x} \pm s$).

Group	NE (ng/mg wet weighting)	DA (ng/mg wet weighting)	5-HT (ng/mg wet weighting)
CG	0.3936 \pm 0.0863	0.7338 \pm 0.1154	0.4457 \pm 0.0279
GL	0.5293 \pm 0.0785**	0.8095 \pm 0.1084**	0.4752 \pm 0.0266
GM	0.3970 \pm 0.0797	0.7443 \pm 0.1173	0.4506 \pm 0.0318
GH	0.3235 \pm 0.0676**	0.6527 \pm 0.1096**	0.4243 \pm 0.0286

**Compared with CG, $P < 0.05$

DISCUSSION

Over the last decade, the expansion of mobile communications has contributed to the general debate about the potential adverse effects of electromagnetic radiation emitted by cellular phones on human brain, in particular to the possible hazard about the embryonic development. A variety of neurological effects have been postulated to electromagnetic radiation, including headaches attributable to use of cellular phones and changes in sleep patterns (Butterfield and Lauderback, 2002).

This study provides two important findings relating to embryonic development in experimental exposure to electromagnetic radiation. Firstly, we demonstrated that long time electromagnetic radiation emitted by cellular phone to pregnant rats could bring about oxidative damage bio-chemically by decreasing the activities of SOD and GSH-Px as well as increasing the content of MDA in brain tissue of fetal rats. Secondly, the electromagnetic radiation exposure to pregnant rats as above could also cause the change of monoamine neurotransmitter levels in fetal rat brain.

This result suggests that a certain dose of intensity microwave radiation can induce brain damage to fetal rats by decreasing the activity of antioxidant enzymes and increasing levels of lipid peroxidation. The similar findings were presented by Ilhan et al. (2004) and Ozguner et al. (2005). Ilhan et al. (2004) found that the activities of SOD and GSH-Px in rat brain tissues decreased with MDA increased after exposure to mobile phone. Ozguner et al. (2005) had consistent results in the study of 900 MHz mobile phone-induced heart tissue damage (Ilhan et al., 2004; Ozguner et al., 2005).

It is possible that electromagnetic radiation affects the mitochondrial membranes to produce large amounts of oxygen radicals. Oxidative stress, referred to as an imbalance between the intracellular production of free radicals and the cellular

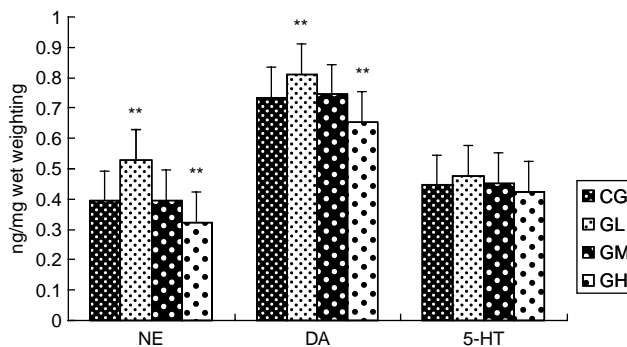


FIGURE 2 The content of NE, DA, and 5-HT in brain tissues of fetal rats for each groups. ** $P < 0.05$ vs. control group.

defense mechanisms, has been implicated in many brain disorders. An excess availability of free radicals, accompanied with a reduction of the capacity of the natural anti-oxidant systems, leads to cellular dysfunction and even cell death. Many studies indicated that EMFs from cellular phone was associated with increased free radical production and lipid peroxidation level in both brain tissue and blood (Irmak et al., 2002).

Oxidative insults, resulting from either an excessive generation of reactive oxygen species (ROS) or the deterioration of antioxidant defense capacity, has been closely linked to the pathogenesis of neuronal dysfunction. It has been demonstrated in numerous studies that ROS was directly involved in oxidative damage of cellular macromolecules such as lipids, proteins, and nucleic acids in tissues. The nervous system is particularly vulnerable to ROS because of the high metabolic rate and deficient oxidant defense mechanisms. And because of the existence of bioelectric field and weak EMFs in cell division, an embryo is very sensitive to the stimuli in an external environment, thus the EMFs from a cellular phone would have a deeper impact on the fetus under certain conditions. Therefore, we mainly carried out the observation on the possible change in fetal rat brain.

Biological systems have many defense mechanisms to protect themselves against the damage of lipid peroxidation. Generally, antioxidant enzymes are increasingly being recognized as important health promoters in conditions such as cardiovascular problems, treatments of many forms of cancer, and aging (Ilhan et al., 2004). The electromagnetic radiation from a cellular phone could produce a large number of oxygen-free radicals and results in the membrane lipid peroxidation through ionization. Antioxidant treatment could prevent or reduce some complications of microwave radiation by getting rid of the free radicals formed in the body during the radiation (Serel et al., 2004). SOD and GSH-Px are important antioxidant enzymes which protect the cells against lipid peroxidation. Superoxide dismutase (SOD), activated by melatonin, is a specific antioxidant enzyme converting superoxide anion (o_2^-) into a reactive oxygen intermediate H_2O_2 . Glutathione peroxidase (GSH-Px) can catalyze the breakdown of H_2O_2 and lipid hydroperoxides to non toxic products. Strong cytotoxic products generated in the end of chain reaction causes the protein cross-linking between the molecular and intermolecular in the fetal rats exposed to mobile phone radiation. These endogenous antioxidant defenses are likely to be perturbed as a result of over production of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue. Particularly, in biological membranes a large number of unsaturated fatty acids susceptible to oxidation in nerve cells of the brain react with oxygen-free radicals, forming new free radicals, and a large number of SOD, GSH-Px are consumed in the process of anti-oxidative damage (Kehrer, 1993; Riley, 1994; Halliwell and Gutteridge, 1984).

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 brain tissue and other organs (Di Mascio et al., 1991). In this study, MDA level increased in brain might be due to the high rate of oxidative metabolic activity and high concentration of readily oxidative membrane polyunsaturated fatty acids of neuronal cells (Hamblin and Wood, 2002).

Neurotransmitters are endogenous chemicals which relay, amplify, and modulate signals between a neuron and another cell. Neurotransmitters are packaged into synaptic vesicles that cluster beneath the membrane on the

presynaptic side of a synapse, and are released into the synaptic cleft, where they bind to receptors in the membrane on the postsynaptic side of the synapse. Release of neurotransmitters usually follows arrival of an action potential at the synapse, but also may follow graded electrical potentials. In the last few years, an increasing number of studies have emerged and indicated that EMFs emitted by cellular phone could affect brain activity. Functions such as sleep, attention, or learning and memory have been also shown to be “mobile phone-sensitive” (Edelstyn and Oldershaw, 2002; Lee et al., 2001; Koivisto et al., 2000a,b; Preece et al., 1999). Hocking and Westerman (2003) reported that radiofrequencies (mainly 2450 MHz) could induce modifications of several neurotransmission systems, in terms of neurotransmitter release or binding properties of receptors.

In this study, we detected three neurotransmitters to investigate potential hazard of electromagnetic radiation from cellular phone to nervous systems. The contents of NE, DA in the GL group increased while decreased in the GH group. The results indicated that fetal rats exposed to high intensity of mobilephone radiation would cause the neurotransmitter metabolic disorders. Mausset-Bonnefont et al. (2004) showed that a strong glial reactivity occurred in different parts of the brain after GSM microwave exposure. Glial reactivity reflects neuronal damage provoked by local over-activation of monoamine neurotransmitters and/or imbalance between excitatory and inhibitory systems. The same result was also found in Zhao’s experimental (918 MHz, 1, 2, 5 mW/cm² for one hour/day for 45 days; Zhao et al., 1999). This result may be because low-intensity radiation from a mobile phone increased the metabolic rate of monoamine neurotransmitters, meanwhile the synthesis of neurotransmitters enhancing reactively by the effect of compensatory. When the intensity of radiation increased, the imbalance between the synthesis and decomposition of neurotransmitters might occurred, resulting in the neurotransmitters decreased. There was also inconsistent result which was reported by Wang et al. (2007). It was possible due to the different parameters of electromagnetic radiation, the different species of animals which caused different tolerances to electromagnetic radiation, and so on.

As a whole, the results obtained in the present study indicate that exposure to EMFs of cellular phone (SAR 0.9 W/kg) could induce modifications in the fetal rat brain, not only oxidative stress system but also neurotransmitters. Because of the widespread use of cellular phones, further investigations with complementary techniques will be necessary to understand the mechanism of relation between EMFs of cellular phone and physiological implications.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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