doi:10.4149/gpb_2010_01_59

The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns

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Abstract. The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed. MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls ($p < 0.001$, Mann-Whitney test). No difference was found in the newborns ($p > 0.05$, Mann-Whitney). There exist very few experimental studies on the effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

Key words: Radiofrequency radiation — Pregnant exposure — MDA — 8-OHdG — Brain

Abbreviations: RFR, radiofrequency radiation; 8-OHdG, 8-hydroxy-2'-deoxyguanosine; dG, deoxyguanosine; MDA, malondialdehyde; IQR, interquartile range

Introduction

Electromagnetic pollution is one of the main environmental health issues caused by the man-made sources like devices for wireless communication. Base stations used for communication through mobile phones are important sources of radiofrequency radiation (RFR). Public concerns have markedly increased due to the oversensitive people such as pregnant women exposed to RFR. Although two of the most influential guidelines, International Commission on Non-Ionizing Radiation Protection (ICNIRP) and Institute of Electrical and Electronics Engineers (IEEE) limit the maximum levels of RFR exposure to general public and workers, they do not comprise pregnant women and their infants.

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Most of the epidemiological and experimental (*in vivo/in vitro*) studies revealed that different frequency ranges of RFR may induce biological responses concerning the cell proliferation, morphology and apoptosis (Repacholi 1998; Marinelli et al. 2004; Zhao et al. 2007), the membrane structure and function (Phelan et al. 1992; Repacholi 1998) and the breakage of DNA (Lai and Singh 1995, 1996, 1997). DNA damage caused by RFR may trigger cancer development (Hardell et al. 2002, 2003), increase the prevalence of infertility due to the effects of hormonal changes on testis (Fejes et al. 2005; Agarwal et al. 2008) and cause fetal loss and developmental malformations (Heynick and Merritt 2003).

Most of the physiological responses of living organism arise from both localized and whole-body average temperature changes based on carrier frequency of RFR. Besides the changes in maternal colonic temperature, fetal body weight is also used as an indicator to determine the threshold of fetal effects induced by RFR or microwave exposure (Berman et al. 1992). It is reported that maternal rectal temperature of

the pregnant mice and the pregnant rats exposed to high levels of microwave energy in 2.45 GHz increased whereas the body weight of the offspring decreased (Berman et al. 1981, 1982, 1984). In the present study, rectal temperatures of both non-pregnant group and non-pregnant-RFR exposed group were measured to determine the temperature changes induced by the exposure to the radiation like GSM (global system for mobile communications) signal.

It has been suggested that one of the interaction mechanisms of RFR with biological matter is the biochemical mechanism that is based on the responses caused by activating secondary chemical messengers such as ions, radicals or molecules (Belyaev 2005). Due to the overproduction of free radicals and deficiency in the amounts of antioxidants, the balance between free radicals and antioxidants is disrupted in favor of the free radicals resulting in oxidative and nitrosative damages which are known as oxidative stress or nitrosative stress (Halliwell and Gutteridge 2000). Oxidative stress may result in severe metabolic dysfunctions, including peroxidation of membrane lipids, depletion of nicotine amide nucleotides, rises in intracellular free Ca^{2+} ions, cytoskeleton disruption and DNA damage. Since repair of almost all of the biomolecules depends on the information coded in the DNA, there is a postulated importance of oxidative DNA damage. 8-hydroxy-2'-deoxyguanosine (8-OHdG) is one of the most frequently used base adducts generated by free radical attacks (Cook et al. 2003). Another metabolic dysfunction of oxidative stress is the peroxidation of lipids. Lipid peroxides, which are formed as a result of complex chain reactions mediated by reactive oxygen species (ROS), are considered to be an important cause of damage to cell membranes. One of the main biomarkers widely used in determination of oxidative destruction on lipids mediated by second messengers is malondialdehyde (MDA) (Nair et al. 1986; Draper and Hadley 1990).

The present study was designed to evaluate the possible biological effects of whole body 1800 MHz GSM-like RFR exposure on brain oxidative DNA damage and lipid peroxidation levels in both non-pregnant and pregnant New Zealand White rabbits and in their newly borns. This study tries to simulate the exposure of non-pregnant and pregnant mobile phone subscribers of the same age during pregnancy and their babies that are exposed to these fields as fetus.

Materials and Methods

Exposure level and quality control

GSM-like signals in 1800 MHz frequency were formed by using a signal generator (Agilent Technologies 8648C, 9 kHz–3.2 GHz) with the integrated pulse modulation unit and horn antenna (Schwarzbeck, Doppelsteg Breitband Horn antenna BBHA 9120 L3F, 0.5–2.8 GHz) in a shielded room. The generated power was controlled by a spectrum analyzer (Agilent Technologies N9320A, 9 kHz–3 GHz) integrated to the signal generator. The signals were amplitude-modulated by rectangular pulses with a repetition frequency of 217 Hz and a duty cycle of 1 : 8 (pulse width 0.576 ms), corresponding to the dominant modulation component of the GSM.

RFR generator provided 0.1 W (20 dBm) during the exposure period. The signal was controlled by means of the spectrum analyzer connected to the signal generator, and NARDA EMR 300 and type 26.1 probe were used for measurement of the output radiation. Measurements were taken during the entire experiment and the data was saved in the computer which was connected to the device *via* fiber optic cable. The evaluated data was 14 ± 0.5 V/m (Figure 1).

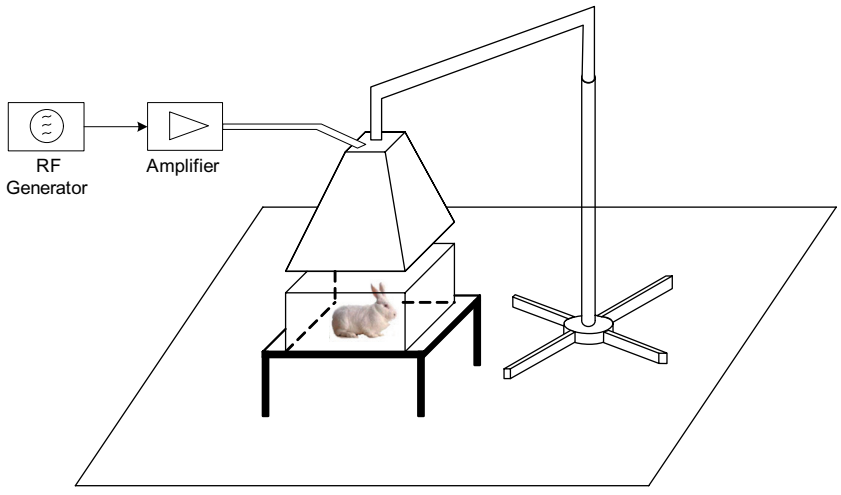

Figure 1. GSM-like RFR exposure system.

Animals

New Zealand White rabbits were obtained from the Laboratory Animals Breeding and Experimental Research Center of Gazi University (13-month-old non-pregnant and pregnant rabbits; $n = 36$). The experimental protocol was reviewed and approved by the Laboratory Animal Care Committee of Gazi University. All the animal procedures were performed in accordance with the approved protocol.

Rabbits were housed under the same conditions in a temperature and humidity-controlled room (20 ± 1°C, 50 ± 10% relative humidity) and 14–16 h light/dark cycle conditions. Except during exposure periods, tap water and standard pelletized food were provided *ad libitum*. Coeval pregnant and non-pregnant rabbits were adapted to the laboratory conditions for five days before the experiment. Quality controls were conducted by the veterinarians to verify that rabbits used in this experiment were healthy at all stages of the experiment. The aim of these controls was to check the health condition of the adult rabbits, condition of the gestation and any malformation or prenatal death of the offspring.

In the breeding process, we selected female rabbits that would get pregnant for the fourth time. Because previous experience of being a mother three times leads to obtain high performance in the number of litter. Besides, mothers are then experienced enough to feed and look after their babies. During the mating process, male ratio was kept constant being one male to 10 female rabbits. Pregnancies were verified by abdominal palpation ten days after the process of mating.

Pregnant and non-pregnant rabbits are exposed to RFR in the same conditions for 7 days after the adaptation period. Exposure period was between 15^{th} and 22^{nd} days of the gestation for the pregnant rabbits. After the exposure, rabbits were left on their own without any intervention until gestation period of pregnant rabbits, approximately 30 days is over.

After birth, only one newborn, not exposed to RFR (max. 2 days) was randomly selected from each litter and killed immediately after the anesthesia. The main reason for investigating the newborns was to observe the possible effects of intrauterine RFR exposure (between 15^{th} and 22^{nd} days of the gestational period when the transition from embryogenesis to organogenesis takes place).

Only one animal was placed in each cage during each RFR exposure period because placing more than one animal in a cage would create a stress factor. The rectal temperatures were measured before and after the exposure by digital thermometer (Elite, Istanbul, Turkey) in both non-pregnant-control and non-pregnant-RFR exposed rabbits.

Experimental design

Non-pregnant and pregnant New Zealand White rabbits were randomly divided into four groups. Animals were kept in plexiglass cage since plexiglass is a non-conductive material that is not affected by RFR.

Group I (non-pregnant-control): Each non-pregnant rabbit in sham-exposed group ($n = 9$) was kept in experimental setup for 15 min/day during 7 days with the device switched-off.

Group II (non-pregnant-RFR exposed): Non-pregnant rabbits in RFR-exposed group ($n = 9$) were exposed individually to 1800 MHz GSM-like RFR for 15 min/day during 7 days.

Group III (pregnant-control): Pregnant rabbits ($n = 9$) were kept in experimental setup for 15 min/day during 7 days with the device switched-off.

Group IV (Pregnant-RFR exposed): Pregnant rabbits in RFR-exposed group ($n = 9$) were exposed individually to 1800 MHz GSM-like RFR for 15 min/day during 7 days.

Newborns ($n = 18$) were also divided into two groups:

Group V (newborns of Group III): Newly born rabbits ($n = 9$) of pregnant-control group.

Group VI (newborns of Group IV): Newly born rabbits ($n = 9$) of pregnant-RFR exposed group. They were exposed to 1800 MHz GSM-like RFR for 15 min/day during 7 days in the intrauterine period (between 15^{th} and 22^{nd} days of the gestational period).

The day after the last exposure, rabbits were anesthetized with ketamine (35 mg/kg, intramuscular) and xylazine (5–10 mg/kg, intramuscular), then killed.

Biochemical analysis

Brain tissues rinsed with ice-cold buffered saline and stored at −30°C (maximum 10 h) for double-blind biochemical analysis. After weighing, the brain was cut into small pieces and then homogenized in four volumes of ice-cold Tris-HCl buffer (50 mmol/l, pH 7.4) by using homogenizer (Disperser T10 basic D-79219, IKA-WERKE, GmbH, Staufer).

MDA levels were analyzed in the brain homogenate. The principle of MDA determination method is based on the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA (Draper and Hadley 1990).

For the measurement of oxidative DNA damage (lesions/10^6 DNA nucleosides), the genomic DNA of brain tissues was extracted by Roche DNA extraction kit, and it was denatured by heating for 3 min at 95°C and then cooled on ice. 100 µl 2 mmol/l desferrioxamine-B mesylate (DFAM) and 20 mmol/l acetate buffer (pH = 5) were added to the denaturated DNA. DNA content was analyzed spectrophotometrically at 260 nm and then hydrolyzed to nucleotides by incubation with 4 µl of 3.3 mg/ml suspension of nuclease P1. The Tris-HCl buffer (pH = 8.5) was added to the mixture and hydrolyzed to the corresponding nucleosides by incubation with calf intestine alkaline phosphatase for 1 h at 37°C. After adding up acetate buffer and 50 mmol/l ethylenedi-

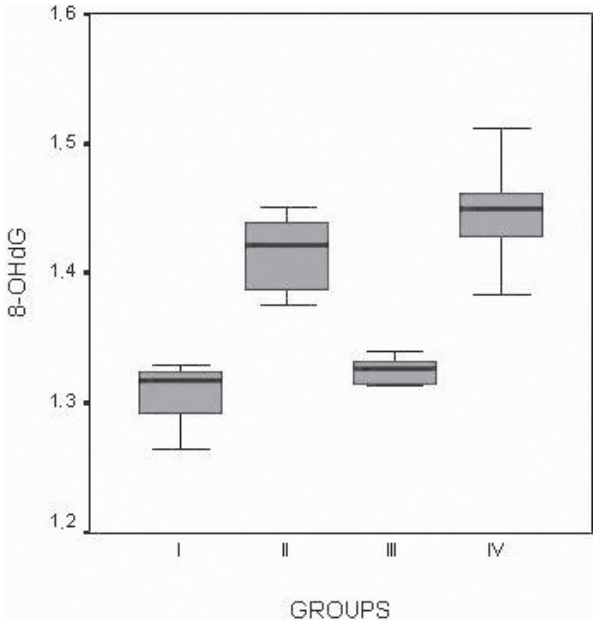

Figure 2. Effects of 1800 MHz GSM-like RFR exposure on 8-OHdG (8-OHdG/10^5 dG) content in the brain of New Zealand White rabbits (n = 9). Subjects were exposed for 7 days, 15 min/day. All values are expressed as median (IQR) values. I, nonpregnant-control; II, nonpregnant-RFR exposed; III, pregnant-control; IV, pregnant-RFR exposed.

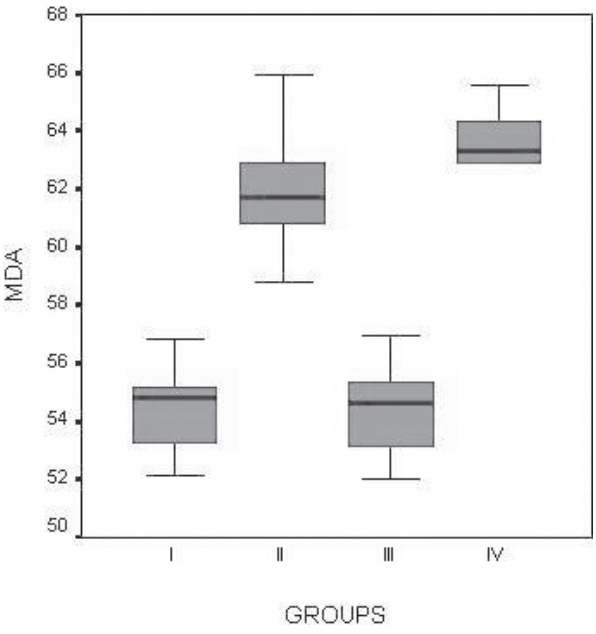

Figure 3. Effects of 1800 MHz GSM-like RFR exposure on MDA (nmol /g wet tissue) content in the brain of New Zealand White rabbits (n=9). Subjects were exposed for 7 days, 15 min/day. All values are expressed as median (IQR) values. I, non-pregnant-control group ; II, non-pregnant-RFR exposed group; III, pregnant-control group; IV, pregnant-RFR exposed group.

aminetetraacetic acid (EDTA) /10 mmol/l DFAM solution, the mixture was filtered through a 0.22 μm Millipore filter unit (UltraFree, Bedford, MA, USA) and then centrifuged at 10 000 × g for 20 min at 4°C. Reverse-phase high pressure liquid chromatography/electrochemical detection (HPLC-EC) was performed as described by Floyd et al. (1986). The DNA hydrolysate was injected onto a Waters C18 reverse-phase column (5 μm, 0.46 cm × 25 cm; Waters Assoc., Milford, MA, USA) at a flow rate of 1 ml/min. The mobile phase was 50 mmol/l phosphate buffer (pH = 5.5) with 5% methanol (Halliwell and Dizdaroglu 1992; Hamilton et al. 1999).

The eluant was monitored at 290 nm for the ultraviolet detection of deoxyguanosine (dG) and at 0.6 V for the electrochemical detection of 8-OHdG. The system was calibrated with authentic dG and 8-OHdG standards (Sigma Chemical, St. Louis, MO, USA). dG had a retention time of 10–12 min and 8-OHdG had a retention time of 8.7–13.8 min. Standards were run after every fifth sample for verification, and the data were expressed as the ratio of 8-OHdG to 10^6 dG.

Statistical analysis

Data analysis was carried out using the SPSS 11.5 statistical package (SPSS, Chicago, IL, USA). The Kruskal-Wallis (non-parametric) test was applied to evaluate differences among all groups while differences between pairs of groups were evaluated by means of the Mann-Whitney test. The results were expressed as median (interquartile range, IQR) values.

Results

Effect of RFR on DNA damage and MDA level in adult female rabbits

It was found that the brain tissue level of 8-OHdG increased in both non-pregnant and pregnant-RFR exposed groups [Group II – median 1.4220 8-OHdG/10^6 dG (IQR 0.0585–0.08) and Group IV – median 1.4490 8-OHdG/10^6 dG (IQR 0.0490–0.13)] with respect to non-pregnant control group [Group I – median 1.3170 8-OHdG/10^6dG (IQR 0.0332–0.06)] (p < 0.001, Mann-Whitney test) (Figure 2). Significant increase was also found in pregnant-RFR exposed group [Group IV – median 1.4490 8-OHdG/10^6dG (IQR 0.0490–0.13)] in comparison to pregnant control group [Group III – median 1.3270 8-OHdG/10^6dG (IQR 0.0215–0.05)] (p < 0.001, Mann-Whitney).

Moreover, MDA levels in the same groups [Group II – median 61.7219 nmol/g wet tissue (IQR 3.8754–7.14) and Group IV – median 63.3184 nmol/g wet tissue (IQR 3.5460–7.49)] were found to increase significantly with respect to non-pregnant controls [Group I – median 54.7823 nmol/g wet tissue (IQR 2.8269–4.70)] (p < 0.001, Mann-Whitney)

(Figure 3). Significant increase was also found in pregnant-RFR exposed group [Group IV – median 63.3184 nmol/g wet tissue (IQR 3.5460–7.49)] in comparison to pregnant control group [Group III – median 54.6298 nmol/g wet tissue (IQR 3.2262–4.94)] ($p < 0.001$, Mann-Whitney).

Effect of RFR on DNA damage and MDA level in rabbit newborns

There was no significant difference in brain tissue 8-OHdG levels of Group VI [median 0.6713 8-OHdG/105 dG (IQR 0.0477–0.06)] and Group V [median 0.6480 8-OHdG/105 dG (IQR 0.0433–0.06)] (Figure 4). Similarly, no difference was found in the MDA levels between newborns of pregnant-RFR exposed [Group VI – median 21.0680 nmol/g wet tissue (IQR 2.2450–3.82)] and newborns of pregnant-controls [Group V – median 19.8710 nmol/g wet tissue (IQR 1.4960–2.70)] (Figure 5).

Effect of RFR on body weight in adult female rabbits

The weights of all experimental groups were measured. While subjects in Group I and Group II were measured after the last day of exposure, Group III and Group IV were measured once the animals gave birth due to the high risk of miscarriage. No significant difference was found between Group I and Group II. However, there were slight decreases between pregnant-exposed and pregnant-controls with respect to non-pregnant-controls, but the differences were not statistically significant ($p > 0.05$, Mann-Whitney) (Table 1).

Effect of RFR on body weight in rabbit newborns

The weights of newborns were measured instantly after birth. No significant difference was found between Group V and Group VI ($p > 0.05$, Mann-Whitney) (Table 2).

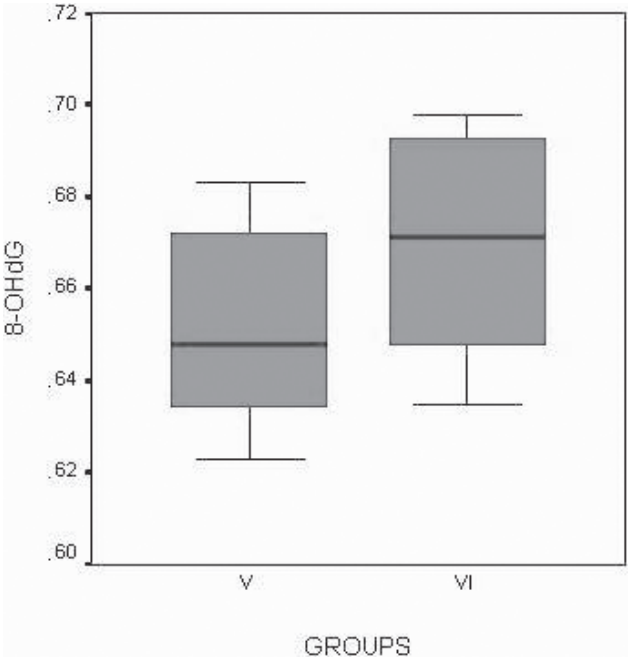

Figure 4. Effects of 1800 MHz GSM-like RFR exposure on 8-OHdG (8-OHdG/10^5 dG) content in the brain of newly born New Zealand White rabbits ($n = 9$). All values are expressed as median (IQR) values. V, newborns of Group III; VI, newborns of Group III.

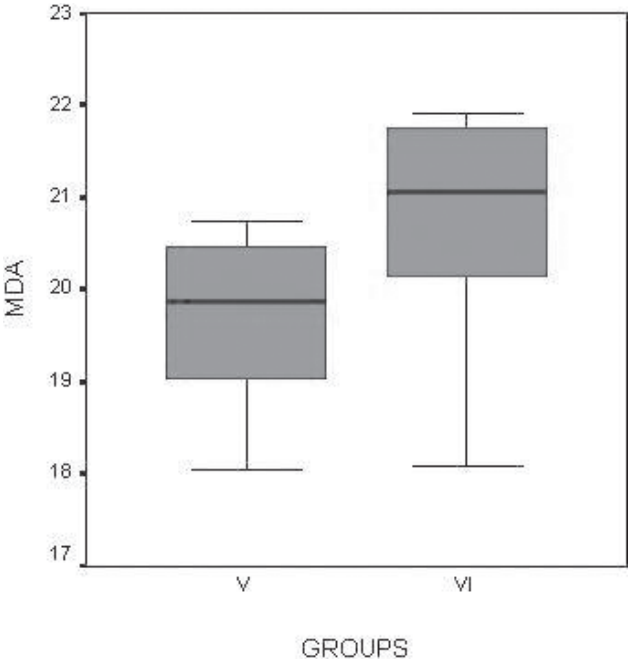

Figure 5. Effects of 1800 MHz GSM-like RFR exposure on MDA (nmol/g wet tissue) content in the brain of newly born New Zealand White rabbits ($n = 9$). All values are expressed as median (IQR) values. V, newborns of Group III; VI, newborns of Group III.

Discussion and Conclusion

Experimental data obtained from this study indicated that 1800 MHz GSM-like RFR exposure of non-pregnant and pregnant rabbits for 7 days (15 min/day) resulted in releasing secondary messengers, such as free radicals, leading to oxidative destruction in lipids and DNA molecules. However, no difference was found in the parameters analyzed in the newborns that were intrauterinely exposed to 1800 MHz GSM-like RFR.

Since the results of relevant clinical and epidemiological studies have been inconsistent, possible health effects related to RFR emitted by mobile phones are still unclear and debated. Health effects of RFR are mainly classified as "ther-

Table 1. The weights of non-pregnant and pregnant New Zealand White rabbits after 1800 MHz GSM-like RFR exposure

Groups	Weight (g)
I. Non-pregnant-control	3825 (792–1020)
II. Non-pregnant-RFR exposed	3900 (575–1150)
III. Pregnant-control	3500 (450–1300)
IV. Pregnant-RFR exposed	3690 (467–1381)

All data are expressed as median (interquartile range, IQR).

Table 2. The weights of newborn rabbits after intrauterine exposure to 1800 MHz GSM-like RFR signals

Groups	Weight (g)
V. Newborns of Group III	59 (21–32)
VI. Newborns of Group IV	62 (23–35)

All data are expressed as median (interquartile range, IQR).

mal" and "non-thermal" effects. Reference levels, namely the maximum level of whole body exposure established by ICNIRP for 1800 MHz RFR are 127.3 V/m for workers and 58.3 V/m for general public. While the current safety standards are based on the thermal effects of RFR in acute exposure, people are chronically exposed to these fields at the non-thermal levels. However, there is still no international safety limit identified specifically for pregnant women. The safety limit for them should be set below the limits for general public or workers in order to protect pregnant women exposed during gestation and baby exposed intrauterinely to RFR. In the present study, the exposure level that we applied (14 V/m) is below the standard levels.

In literature, there are studies on the thermal effects of RFR exposure increasing maternal rectal temperature during pregnancy period and the anatomic and physiological changes in offspring related to intrauterine exposure induced by the temperature difference in mother (Berman et al. 1981, 1982, 1984). Female Sprague-Dawley rats were exposed to 2450 MHz (CW) microwave radiation at incident power densities of 28 mW/cm^2 and 40 mW/cm^2 for 100 minutes everyday from the 6th to 15th day of gestation (Berman et al. 1981, 1984). These authors reported that average colonic temperatures increased in both levels of exposure, but mean fetal body weight was reduced after exposure to higher level of power density (40 mW/cm^2). They also reported that maternal rectal temperatures of hamsters exposed to 30 mW/cm^2 for 100 minutes a day from the 6th to 14th day of gestation increased by 1.6°C. They found that exposure increased the fetal resorptions, decreased fetal body weight and skeletal maturity. They concluded that the hamster fetus may be more susceptible to microwave radiation than the mouse (Berman et al. 1982). In our study, we made similar observations. Pregnant rabbits were exposed to 1800 MHz GSM-like RFR for 15 min everyday from the 15th to 22nd days of gestation which corresponds to the development period of embryo and transition from embryogenesis to organogenesis. We aimed to observe the rectal temperature changes in pregnant rabbits in order to determine the possible intrauterine effects of RFR at the very beginning of the experiment. However, rabbits are very sensitive animals especially when they are pregnant. They have a tendency to miscarriage when they are stress out. Since measuring rectal temperature may stress the pregnant animals out, we measured the temperature change in only non-pregnant group. After last exposure period, adult rabbits were weighed (Table 1) and a slight decrement in the maternal weight was observed.

In addition to this, no deformation on the extremities and the skeletons of the newborn rabbits was observed during the veterinary controls. Low level of radiation, that rabbits were exposed to, may be one of the reasons for these evidences. Also, the thermoregulatory mechanisms of rabbits may be another factor that is sufficient to compensate slight changes in body temperatures by increasing respiration rate and ear lobe temperature (Fayez et al. 1994). It is suggested that RFR exposure increases the physiological, neurological, cognitive and behavioral changes; it also induces, initiates and promotes carcinogenesis (Gandhi 2005). Higher proportion of DNA damage may be caused by an increase in the formation of free radicals and a deterioration in DNA repair capability and/or efficiency (Kundi et al. 2004). In this manner, free radicals can be classified as one of the most important risk factors which may influence structural biomolecules within the cell. In this study, we discussed that 1800 MHz GSM-like signals may induce DNA base modification through increasing the activation of free radicals.

Recent studies conducted on oxidative stress induced by RFR emitted from mobile phones revealed that it may be caused by an increase in the release of free radicals. Ilhan et al. (2004) determined that mobile phone exposure (1 h/day, 7 days) may induce oxidative stress mediated by ROS in brain tissues of the rats. Moreover, Oktem et al. (2005) demonstrated that exposure to mobile phone irradiation during 30 min/day for 10 days increased tissue MDA levels by suppressing antioxidant enzyme activities. Furthermore, Meral et al. (2007) revealed that RFR generated from cellular phone (12 h/day, 30 days) may produce oxidative stress by increasing MDA levels of brain tissues in guinea pigs. On the contrary, Irmak et al. (2002) reported that mobile phone radiation did not change the MDA level (30 min/day, 7 days). Likewise, in this study, we found that whole-body 1800 MHz GSM-like RFR exposure for 15 min/day for a week may affect lipid peroxidation by increasing MDA levels in non-pregnant and pregnant rabbits.

Lai and Singh evaluated DNA damage by measuring DNA strand breaks in brain tissues of rats exposed to 2450 MHz

microwave radiation at 0.6 W/kg for 2 h a day. (Lai and Singh 1995, 1996, 1997). Results of these studies showed that single and double strand breaks occurred after the exposure. In the same manner, we suggested that the base modification in DNA molecules (8-OHdG) can be induced by the attacks of hydroxyl radical (˙OH) in both non-pregnant and pregnant animals under RFR exposure.

Although brain tissue of pregnant and non-pregnant rabbits is affected by RFR, there is no effect on 8-OHdG and MDA level analyzed in the newborns. The reason why these parameters did not change in the newborns may be explained by the phenomena of depth of penetration. As RFR propagates in the tissue medium, energy is absorbed by the tissue, resulting in a progressive reduction of RFR as it advances in the tissue. This reduction depends on the depth of penetration, which is the distance in which the power density decreases by a factor of e^2 (Polk and Postow 1986). Since the fetus is an inner tissue, more so than the brain of the mother, RFR energy may be reduced to such an extent that fetus is not affected.

Consequently, there hasn't been any research published yet on GSM-like radiation on oxidative DNA and lipid damage in the brain tissue of pregnant animals and their offspring. With this perspective, our results may constitute a reference for the future pregnancy studies. Moreover, it would be beneficial to increase the number of these studies for establishing international standards for the protection of pregnant women under RFR exposure.

Acknowledgements. This work was supported by grants from Gazi University Research Foundation, Project No. 01/2005-07. RFR measurements were performed with the devices granted from Gazi University Research Foundation, Project No. 01/2003-62. We would like to acknowledge our heart-felt thanks to Bilge Has Bıyıklı for her dedicated efforts in editing of English usage and grammar of the manuscript.

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Received: April 18, 2009
Final version accepted: September 17, 2009