Assessment of DNA Sensitivity and Heat Stress Protein Response (HSP70) in Male Wistar Rat Blood After Exposure to Microwave Radiation

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Abstract: Because of the increasing use of mobile phones, the possible risks of non-ionizing radiofrequency of electromagnetic fields (RF-EMF) adverse effects on human health was evaluated. The present study aims to assess possible DNA damaging effects induced by microwave radiation of mobile phone type in male Wistar rat blood in *vivo*. A number of 30 male Wistar rats (118±20g) was divided into 3 groups (10 animals in each group) exposed for (15, 30 and 60 min.) to a working Global System for Mobile Communication (GSM) cell phone rated at a frequency of 900 MHz, at non-thermal specific absorption rate (SAR) of 2.9 W/Kg. Concurrent control animals (n=10) were also included in the study. After the exposure periods five animals from each group were sacrificed immediately while the other five animals were sacrificed after 7 days (recovery period). DNA sensitivity in rat blood leukocytes was assessed by using the alkaline comet assay method. The heat shock protein stress response (HSP70) in serum samples of the rats was also investigated. The results showed significant increased DNA damage in blood leukocytes after the exposure times 15 and 30 min and after the three exposure times of the recovery period (7 days) as detected by the comet assay method. Serum HSP70 levels were also significantly increased in the exposed animals and in the animals at the recovery period as compared to the control animals. The present study indicates that RF-EMF represents a potential DNA-damaging hazards and using the alkaline comet assay is a sensitive tool in the measurement of DNA damage after exposure to 900 MHz microwave radiation in *vivo*. The increased HSP70 stress response to RF-EMF exposure might involved in protecting cells from DNA damage induced by microwave radiation. [Journal of American Science. 2010;6(10):218-225]. (ISSN: 1545-1003).

Key words: comet assay – DNA damage – microwave radiation – HSP70

1. Introduction

Microwave radiation is a type of non-ionizing electromagnetic radiation present in the environment and of potential threat to human health (Croft *et al.,* 2002). Today, non-ionizing radiation has increasingly been used in industry, commerce, medicine and for private purposes, especially in mobile telephone usage. Although the average exposure levels are low compared to the exposure limits, there is a growing public concern about the potential hazard of exposure to these frequencies for human health (BreckenKamp *et al.,* 2003; ICNIRP, 2004; Jauchem, 2008 and Garaj-Vrhovac *et al.,* 2009).

Genotoxic studies on microwave radiation in *vivo* and in *vitro* have yielded contradictory and often intriguing experimental results (Vijayalaxmi and Obe, 2004; Verschaeve, 2005). Some reports suggest that exposure of human cells to radiofrequency radiation does not result in increased genetic damage (Vijayalaxmi *et al.,* 2000; McNamee *et al.,*2003). In addition a number of studies have been conducted on animal models. These studies have also given contradictory results regarding exposure to microwave radiation (Witt *et al.,*2000; Vijayalaxmi *et al.,*2003; Paulrai and Behari, 2006).

Epidemiological studies have demonstrated that, there is a link between microwave exposure and excess of cancer, leukemia and brain tumors (Maskarinec *et al.,* 1994; Szmigielski, 1996). Other investigated health outcomes include spontaneous abortions, lenticular changes, neurological and sensitivity reactions, haematological or chromosome changes occurring in certain populations exposed to microwave radiation (Zotti-Martelli *et al.,* 2000; Trosic *et al.,* 2004). On the other hand, there is a range of studies showing that radiofrequency radiation can induce genetic alteration after exposure to microwave radiation (Garaj-Vrhovac *et al.,* 1990 and 2009; Fucic *et al.,* 1992; Maes *et al.,* 1993; Zotti-Martelli *et al.,* 2000; and Tice *et al.,*2002).

The comet assay is now a well-established genotoxicity test for the estimation of DNA damage at the individual cell level both in *vivo* and in *vitro*. The comet assay has widely been used to detect primary biological effects on the level of DNA molecule in human and animal cells exposed to several environmental or occupational substances

(Collins *et al.,* 1997; ESCODD, 2003; Kumaravel *et al.,* 2009 and Garaj-Vrhovac and Oreščanin, 2009). Therefore, this method makes it possible to evaluate the level of primary DNA damage even after short-term of exposure to irradiation.

Living cells have mechanisms to maintain homeostasis and the activation of heat shock proteins (heat shock/stress protein) as a normal defense response to cellular stress (Santoro, 2000; Nollen and Morimoto, 2002) such as the non-thermal response to microwave radiation (Goodman and Blank, 1998 and 2002). Also, certain previous studies have shown stress response proteins and particularly HSP70 to be activated by the electromagnetic radiation emitted from mobile phones (de Pomerai *et al.,* 2000 and Weisbrot *et al.,* 2003).

The aim of the present study, was to assess possible DNA damaging effects induced by 900 MHz microwave radiation of mobile phone type in male Wistar rat blood in *vivo*. For this purpose the alkaline comet assay method as a sensitive tool in detecting primary DNA damage is used. In addition, the cell stress response to microwave radiation was determined by measuring the HSP70 in blood of male Wistar rats.

2. Material and Methods

Animals

Male Wistar rats weighing about 118±20g were obtained from Helwan Farm for Vaccine and Biological Preparations. The animals were housed in cages 10 animals in each for one week before the beginning of the experiment. The animals were maintained on 12h dark/light cycle and were given food and water *ad libitum*.

Method of exposure

After the acclimation period, animals were randomly divided into four groups, 10 animals per group. The 1st group was used as a control group. The 2nd, 3rd,and 4th groups were exposed to EMF for 15, 30 and 60 minutes respectively. Five animals from each group were sacrificed immediately after exposure while the rest of the animals were sacrificed after a recovery period of 7 days. Control animals were housed in separate cages with the same conditions as the exposure groups but without power input.

During irradiation, each animal was placed in its own Plexiglas cage (25 cm x 7.5 cm x 7.5 cm). For EMF exposure, a cell phone in the "on" mode was placed with its antenna over the center of the cage. The cell phone was manufactured by Nokia (model 6300 type RM-217, GSM 900MHz). The power density of the field was 0.35 W/m^2, corresponding to a whole-body specific absorption rate (SAR) of 2.9 W/Kg. Control animals were performed similarly as the exposed group and the cell phone is in the switched off˝ mode.

Blood sampling

After the exposure period of 15, 30 and 60 min blood samples were collected immediately and after recovery period of 7 days. One rat at a time was anesthetized by placing it in a glass jar containing cotton dipped in anesthetic ether. The rats were then decapitated and blood samples were collected from dorsal aorta under sterile conditions in heparinzed tubes and kept at -4°C until analysis. A part of the blood (2ml) were centrifuged for 15 min at 4000 r.p.m. and serum was stored at -4°C until analysis.

Single cell gel electrophoresis (comet assay)

To measure the potential DNA damaging effect of microwave radiation in single Wistar rat leukocytes, the comet assay was carried out as described by Jaloszynski and Szyfter (1999). A total of 50 cells were examined in this experiment. For each cell, the length of DNA migration (comet tail length) was measured in micrometers from the center of nucleus to the end of the tail. The percentage of damaged DNA concentration in the comet tail was determined by measuring the total intensity of ethidium bromide fluroscence in the cells, which was taken as 100% and determining what percentage of this total intensity correspond to the intensity measured only in the tail.

Determination of serum HSP70 levels

The levels of HSP70 (pg/ml) in the serum samples were determined according to the method described by Oc *et al.* (2008) using ELISA Kit (DUOSEt®IC, US). One hundred µL of each 1:5 (v/v) diluted serum samples and prepared standards were applied to each well precoated with a mouse monoclonal antibody specific for inducible HSP70, the plate was sealed and incubated at room temperature for 2 h on an orbital shaker. After washing six times with wash buffer, the captured HSP70 was detected by 100 µL HSP70-specific, biotinylated rabbit polyclonal antibody, the plate was sealed and incubated at room temperature for 1 h on an orbital shaker. After washing six times with wash buffer, the biotinylated detector antibody was subsequently bound by 100 µL avidin-horseradish peroxidase conjugate. The colour was developed by an addition of 100 µL of tetramethylbenzidine substrate and allowed to react for 15 min. To terminate reaction, 100 µL stop solution was added and the intensity of the colour was measured at 450 nm.

Statistical Analysis

Data were expressed as a mean ± standard error (SE). Differences between the control and treated groups were tested with unpaired student t-test. Statistical differences between control and exposed rats are as follows *P<0.05, **P<0.01 and ***P0.001.

3. Results

The results of the comet assay parameters (mean tail length and mean DNA%) as well as the mean serum levels of HSP70 performed on rat blood at the three different times of exposure to 900 MHz EMF as compared to that of the control animals are summarized in (Table 1)and (Fig.1).

By comparing the values of mean tail length of the comet assay of the exposed and control animals, it is clear that, mean ±SE of tail length of the comet assay showed significantly increased levels (P<0.05 and P<0.00) after 15 and 30 min. of exposure respectively (Fig.1 B). While, the mean tail length of the comet assay showed insignificantly increased values after 60 min. of the exposure period.

On the other hand, the damaged DNA% showed significantly (P<0.05) and (P<0.01) increased levels after 15 and 30 min. of the exposure period, while it increased insignificantly after 60 min. of the exposure period.

After exposing male Wistar rats to 900 MHz EMF for (15, 30 and 60 min.), the mean levels of serum HSP70 increased significantly in all the three times of exposure (Table 1).

When comparing both comet assay parameters (mean tail length and damaged DNA%) in the mobile phone exposed rats and the control ones after 7 days recovery period (Table 2) and (Fig.2), it showed significantly increased levels (P<0.001 and P<0.01) after the three exposure times (15, 30 and 60 min.) respectively (Fig.2 C), except for the damaged DNA% at the exposure time 30 min. which showed insignificantly increased levels as compared to the control animals.

The mean levels of serum HSP70 increased significantly (P<0.001 and P<0.05) after the exposure times 15, 30 and 60 min in animals sacrificed immediately and after the recovery period (Table 2).

Table 1. Comet assay parameters (mean tail length and mean % of DNA damaged ±SE) and mean ±SE of HSP70 levels performed on rat blood at different exposure times to 900 MHz microwaves as compared to the control group.

Parameter	control	15 min	30 min	60 min
Tail length(µm)	0.645±0.06	*2.752±0.28	***2.085±0.13	1.456±0.35
% of DNA damaged	0.414±0.05	*1.410±0.13	**1.321±0.15	0.990±0.58
Hsp70(Pg/ml)	115.630±0.54	***156.188±0.97	***126.908±0.98	*123.859±2.06

Results are means ± SE of 5 animals.
Statistical differences between control and exposed rats as follows: * P< 0.05 (significant), ** P<0.01 (highly significant), and *** P<0.001 (more highly significant).

Table 2. Comet assay parameters (mean tail length and mean DNA% ±SE) and mean ±SE of HSP70 levels performed on rat blood at different exposure times to 900 MHz microwaves and kept for 7 days recovery period as compared to the control group.

Parameter	control	15 min	30 min	60 min
Tail length(µm)	0.820±0.12	***7.998±0.23	**3.269±0.23	**4.249±0.50
% of DNAdamaged	0.520±0.07	***3.383±0.07	1.615±0.41	*.2.351±0.44
Hsp70(Pg/ml)	115.636±0.54	***173.974±05.26	*120.734±1.32	***171.877±4.50

Results are means ± SE of 5 animals.
Statistical differences between control and exposed rats as follows: * P< 0.05 , ** P<0.01, and *** P<0.001

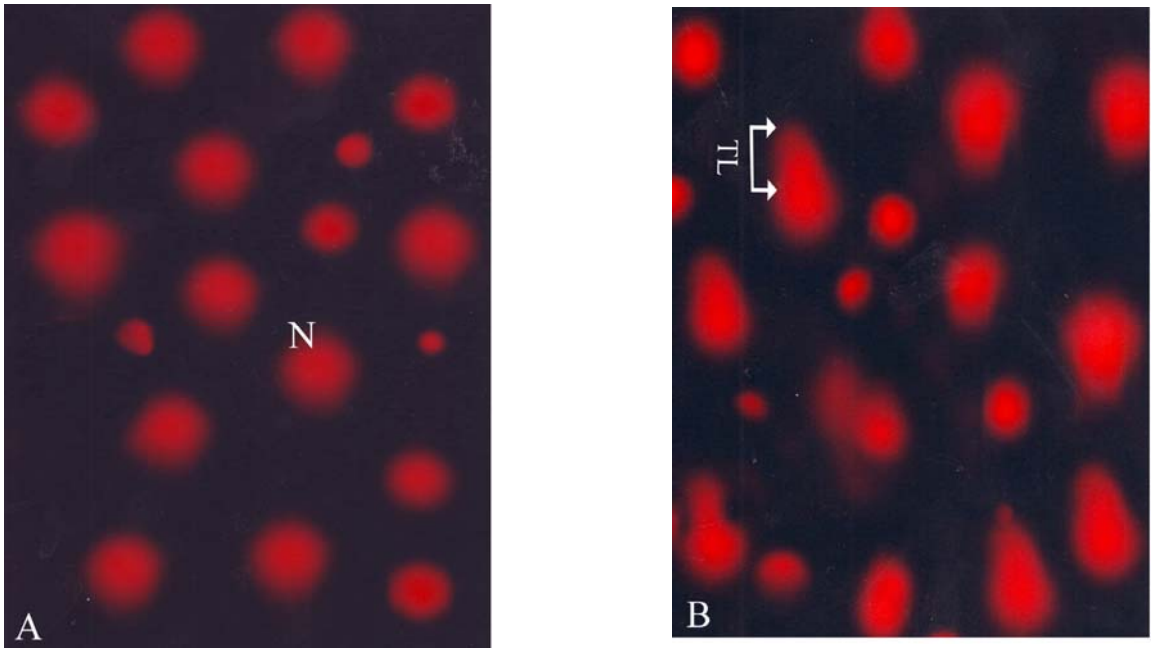

Fig.1. Photograph of male Waster rat leukocytes showing DNA damage (tail length) after exposure to 900 MHz microwave radiation at three different times (15, 30 and 60 min.) as compared to the control group. (A) Leukocyte nuclei from control as established by comet assay method. (B): Damaged spot of DNA. N= Normal nuclei. TL= Comet tail length of migrated DNA.

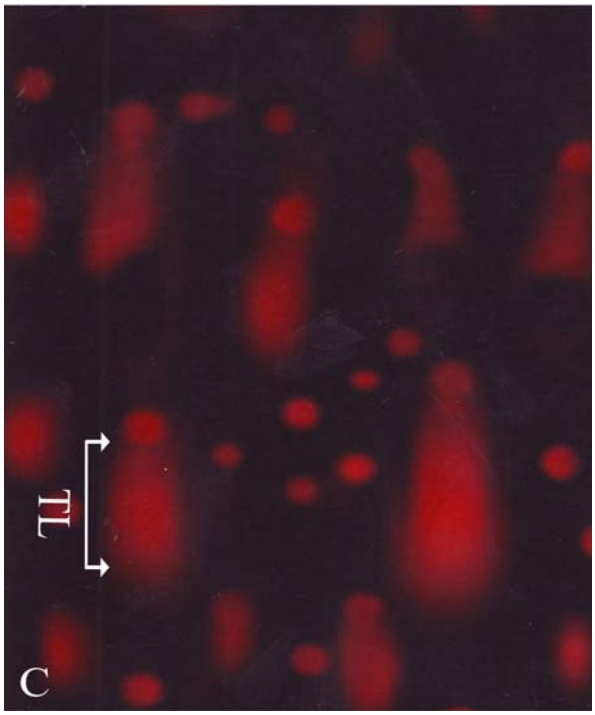

Fig.2. Photograph of male Waster rat leukocytes sacrificed after 7 days recovery period showing DNA damage (tail length) after exposure to 900 MHz microwave radiation as compared to the control group time. (C) Strong damaged spot of DNA. TL= comet tail length of migrated DNA.

4. Discussion:

A large number of experimental and epidemiological studies have been carried out to elucidate the possible health hazards associated within human exposure to RF-EMF. Many studies have provided evidence suggesting that EMF with relatively low intensity are capable of interacting with many molecular, cellular and systemic processes associated with carcinogenesis and teratogenesis (Juutilainen and Lang, 1997). Biologically detri mental effects of the EMF has not yet been sufficiently explored, and the existing data are often contradictory. Because DNA damage is closely related to every aspect of physical and pathological activity of cells, one of the most active areas RF-EMF investigation is the assessment of direct and indirect effects on DNA (Brusick *et al.,* 1998). The single-cell gel electrophoresis (comet assay) is the simplest and most sensitive method that can measure and identify DNA damage at the cellular level (Tice *et al.,*2000). This technique has been widely used for investigations of the possible DNA damage induced by RF-EMF but the results have been contradictory.

In the present study, the alkaline comet assay method was used to test whether microwave radiation can induce DNA damage in male Wistar rat leukocytes after in *vivo* exposure to 900 MHz microwave radiation for 15, 30 and 60 min of exposure. The results showed a significant increase in the comet assay parameters after the exposure times (15 and 30 min) which indicate the presence of DNA damage. Thus the results of the present study demonstrated that the alkaline comet assay can be successfully applied to study microwave-induced DNA damage in rat leukocytes. By using the alkaline comet assay in exposed animals, a lot of DNA single-strand breaks and alkali-labile sites were detected. Similar results were reported by Lai and Singh (1995 and 1996) on rat brain cells immediately and at 4 h of acute exposure to RF-EMF. Their results indicated that acute exposure to microwave radiation at an average body SAR of 1-2 W/Kg caused a significant increase in both single and double-strand DNA breaks and suggested that this could be due to either a direct effect on the DNA and/or an effect of the radiation on DNA repair mechanisms. However, the study of Malyapa *et al.*(1998) did not obtain the same results as Lai and Singh. The poor replicability and contradictory findings may result from the different specifications of EMF, both of which influence the interactions with living cells (Roti *et al.,* 2001). The ability to repair DNA lesions is a ubiquitous defense mechanism that is essential for cell survival and the maintenance of cell functions. Different cells have different DNA repair capacities due to genetic differences (Schmezer *et al.,* 2001).

It should be emphasized that the type of DNA damage measured by alkaline comet assay is rather continuously and efficiently repaired. Therefore, the increased DNA damage observed in the present study after one week of exposure at the different three times is as a result of equilibrium between damaged infliction and repair. It is known that DNA single-strand breaks are rapidly repaired; however, other lesions such as oxidized bases, may persist longer and be misrepaired (Collins *et al.,* 1997). On the other hand, apurinic/apyrimidinic sites (alkali-labile sites) may not be readily repaired and without DNA replication, they may prove to be "silent" lesions (Gichner *et al.,* 2000). It was reported that DNA damage accumulates with time and its repair capacity decreases over time. When DNA damage is not repaired or is improperly repaired, biological effects result. Although majority of DNA lesions are repaired in few hours and days after their infliction, a part of DNA damage induced persisted over time. It can be considered that this elevated level reflects an accumulation of non-repaired DNA damage.

Extensive damage to DNA can lead to cell death. Large number of cells dying can lead to organ failure and death for the individual. Damaged or improperly repaired DNA may develop into cancers. Data on the possible cancer-related effects of microwave radiation are still controversial. However, it is possible that in complex cellular processes involved in carcinogenesis it could have co-carcinogenic effects (Verschaeve and Maes, 1998).

The results of the present study are in agreement with those of (Gichner *et al.*,2000; Trosic *et al.,* 2002 and 2004; Zotti-Martelli *et al.,* 2005; Lixia *et al.,* 2006; Garaj-Vrhovac and Oreščanin, 2009 and Garaj-Vrhovac *et al.,* 2009).

Cells respond to abnormal physiological conditions by producing protective heat shock (or stress) proteins. Heat shock proteins are an important group of cell response proteins. They act primarily as molecular chaperones to eliminate unfolded or miss-folded proteins, which can also appear from cellular stress. This stress response can be induced by many different external factors including temperature, oxidative stress, ionizing and non-ionizing radiation (Ramage and Guy, 2004). It has been reported that HSP70, one of the most studied HSP families is induced with exposure of human epithelial cells to GSM signal of 960 MHz at a SAR of 0.0021 W/Kg for 20 min (Kwee *et al.,* 2001).

Some investigators described an increased HSP level after RF-EMF exposure (de Pomerai *et al.,* 2000; Leszczynski *et al.,* 2002), however, these results are controversial, because of some other negative findings (Cotgreave,2005). The present study indicated that there was a significant increase in

serum HSP70 after the three times of exposure to RF-EMF at SAR 2.9 W/Kg. This increase was persisted also after one week of exposure. Thus the current study conformed a cellular protective response as a result of microwave exposure. Fritze *et al.*(1997) examined the levels of HSP70 m-RNA in rat brain after exposure of animals to EMF from mobile phones and found that SAR of 7.5W/Kg induced HSP70 m-RNA in cerebellum and hippocampus. However, there is also a contrasting reports that radiofrequency radiation were not able to induce the HSP70 response and others speculated that temperature increase not microwave radiation may cause the induction of heat shock gene expression (de Pomerai *et al.,* 2000 and Dawe *et al.*,2006). So, the increase of HSP70 observed in this study might be involved in protecting the cells from DNA damage induced by microwave radiation of the mobile phone exposure.

The results of the present study, are in agreement with those of Fritze *et al.*(1997); Danilles *et al.*(1998); de Pomerai *et al.*(2000); Kwee *et al.*(2001) Lixia *et al.* (2006) and Simkó *et al.* (2006).

In conclusion, the results obtained in the current study demonstrate the presence of DNA damage and heat shock response in male Wistar rat blood due to acute non-thermal exposure to 900MHz RF for GSM. The results also indicated that the alkaline comet assay, as a reliable biomarker of exposure, can be successfully applied in the study of DNA-damaging effects. From a public health standpoint, it is important to re-evaluate the present RF-EMF safety limits proposed by the International Commission on Non-Ionizing Radiation Protection (ICNIRP). Moreover, longer and chronic exposures should be tested to see if the effect could evolve in a permanent effect on the cells or not.

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