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LFS-11490; No of Pages 8

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ScienceDirect
Life Sciences xx (2006) xxx–xxx
Life Sciences
www.elsevier.com/locate/lifescie

Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring

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Received 6 December 2005; accepted 17 August 2006

Abstract

Mobile telephones and their base stations are an important ultra high frequency-electromagnetic field (UHF-EMF) source and their utilization is increasing all over the world. Epidemiological studies suggested that low energy UHF-EMF emitted from a cellular telephone may cause biological effects, such as DNA damage and changes on oxidative metabolism. An in vivo mammalian cytogenetic test, the micronucleus (MN) assay, was used to investigate the occurrence of chromosomal damage in erythrocytes from rat offspring exposed to a non-thermal UHF-EMF from a cellular phone during their embryogenesis; the irradiated group showed a significant increase in MN occurrence. In order to investigate if UHF-EMF could also alter oxidative parameters in the peripheral blood and in the liver – an important hematopoietic tissue in rat embryos and newborns – we also measured the activity of antioxidant enzymes, quantified total sulfhydryl content, protein carbonyl groups, thiobarbituric acid-reactive species and total non-enzymatic antioxidant defense. No significant differences were found in any oxidative parameter of offspring blood and liver. The average number of pups in each litter has also not been significantly altered. Our results suggest that, under our experimental conditions, UHF-EMF is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis through an unknown mechanism.
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Keywords: Chromosomal damage; Cellular phone; Embryogenesis; Micronucleus; Rat offspring; Oxidative stress; Erythrocytes; Liver

Introduction

The use of mobile phones is currently one of the fastest growing technological developments. The close proximity of the antenna of such device to the user's body has raised diverse concerns about the biological interactions with EMF and the direct biological effects which such exposure could originate (Crumpton and Collins, 2004; Lönn et al., 2004; Mild et al., 2003; Szmigielski, 1996; Wertheimer and Leeper, 1979). Recent in vitro and in vivo studies observed the occurrence of DNA damage (Lai and Singh, 1996; Wolf et al., 2005; Zmyślony et al., 2000), as well as micronucleus (MN) generation, which is a well-accepted index for genotoxicity evaluation, after the EMF exposure (Cho and Chung, 2003; Tice et al., 2002; Trosic et al., 2004; Zotti-Martelli et al., 2000).

However, it is not clear how UHF-EMF interacts with living systems. Some authors pointed out a possible role of oxidative

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doi:10.1016/j.lfs.2006.08.018

Please cite this article as: Amâncio Romanelli Ferreira et al., Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence..., Life Sciences (2006), doi:10.1016/j.lfs.2006.08.018.

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stress in this process, and proposed mathematical models explaining how weak electromagnetic fields could impair radical recombination, thus increasing free radicals generation (Timmel et al., 2001; Grissom, 1995). In agreement with this hypothesis some authors suggested that UHF-EMF might also increase free radicals formation, based on the assumption that reactive oxygen species (ROS) are implicated in several types of tissue injury (Ilhan et al., 2004; Lee et al., 2004; Oktem et al., 2005; Regoli et al., 2005; Yokus et al., 2005; Zmyślony et al., 2004). The major ROS to be considered as potentially harmful are the superoxide anion ($O_2 \cdot^-$), which is generated mainly in mitochondria; hydrogen peroxide (H_2O_2), generated by the action of the enzyme superoxide dismutase (SOD) upon $O_2 \cdot^-$; hydroxyl radicals (OH.) produced from H_2O_2 through the transition metal-catalyzed (most frequently iron or copper) Fenton chemistry; and peroxynitrite (ONOO.), which is generated by the reaction of $O_2 \cdot^-$ with nitric oxide (NO.). ROS are scavenged by SOD, and also the enzymes glutathione peroxidase (GSH-Px) and catalase (CAT) (Halliwell and Gutteridge, 1999).

It is known that proliferating-tissues, e.g. hematopoietic tissue, are more susceptible to noxious stimulus (Trosic et al., 2004). A growing body of evidence has been suggesting that different EMF irradiation patterns are able to cause alterations in circulatory parameters of cerebral and vascular peripheral system of mice and humans (Braune et al., 1998; Prato et al., 1994; Salford et al., 2003). Other studies also reported an increase in the MN incidence in vitro (Tice et al., 2002) and in vivo (Trosic et al., 2004). In agreement with that, Babbitt et al. (2000) observed an induction of hematopoietic neoplasia in vivo by EMF.

The aim of the current study is to investigate whether UHF-EMF is able to induce MN formation in erythrocytes of rat offspring whose mothers were irradiated during the pregnancy, and to study the presence of imbalances in free radical metabolism of rat offspring liver and blood.

Material and methods

Animals

All experiments were performed in strict compliance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publication no. 85-23, revised 1996). Adult pregnant Wistar rats (90–110 days old; 200 g–250 g) were used in this work. Prior to and during exposure the rats were housed into individual plexiglas cages (40.5×33.5×17.5 cm), under a 12-h light/dark cycle at 23±0.5 °C, with water and commercial balanced diet provided ad libitum.

UHF-EMF exposure conditions

Pregnant rats were placed into experimental cages in the first day of pregnancy. Beginning of the pregnancy was determined by daily evaluation of sperm presence in vaginal smear by optical microscopy analysis (ZEISS Axiophot, magn, ×100). A total of 10 pregnant rats have been used, four rats in control group and six rats in UHF-EMF irradiated group. Control group was submitted to the same conditions used in the UHF-EMF group, but without cellular phone. Pregnant rats were irradiated from 5.30 pm until 2.00 am (each day) for a total of 8.30 h per day, since the day of sperm detection (pregnancy start) until offspring birth. The plexiglas cage was placed 29.5 cm away from the UHF-EMF source (cellular phone antenna) in its maximum radiation direction, which correlates to the recipient center. Both the cellular phone and the plexiglas cage were placed inside an aluminum Faraday cage (101.5×64.0×38.0 cm; 0.5 cm mesh), as shown in Fig. 1. The EMF environmental background was 0.0–0.3 V/m. The Faraday cage containing the control animals was kept 40.5 cm away from the second Faraday cage containing the UHF-EMF source. The two Faraday cages were placed in a wood shelf and had their places switched to each other daily, in order to standardize light conditions during the pregnancy. The electric field measured inside the control cage did not differ from the background level (0.0–0.3 V/m). An analogue mobile phone was used as the source of UHF-EMF radiation (600 mW peak; 834 MHz; 26.8–40 V/m; vertical polarization). The specific absorption rate (SAR) can be approximately calculated according to the formula (ICNIRP, 1998; IEEE/ANSI, 1992):

$$\mathrm{SAR} = \frac{\sigma}{\rho}|E_{\mathrm{RMS}}|^2[\mathrm{mW/g}], \tag{1}$$

where E_{RMS} is the root mean square value of the electrical field, σ is the mean electrical conductivity of the tissues and ρ is the mass density. The parameters for the rats were measured according to Peyman et al. (2001). SAR values between 0.55

Fig. 1. UHF-EMF exposure set-up. In this picture, the UHF-EMF Faraday cage is over the control Faraday cage. Inside the UHF-EMF Faraday cage, the plexiglass cage and the analogue cellular phone can be seen.

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and 1.23 W/kg were calculated using (1), assuming: $\sigma=0.8$ S/m (average liver value) and $\rho=1040$ kg/m^3. This range is below the proposed limits to prevent thermal effects, 2 W/kg (ICNIRP, 1998). International guidelines limit the local SAR to a maximum of 2 W/kg (ICNIRP, 1998) or 1.6 W/kg (IEEE/ANSI, 1992). Since exposure is ≤ 1.23 W/kg, the applied microwave power density has been reported not to affect rat body temperature (ICNIRP, 1998). In this regard, the exposure level used in this paper can be considered as non-thermal.

The field intensity was measured with a radiofrequency spectrum analyzer (Anritsu MS2711, Japan), and electromagnetic field meters (EMR30/EMR300, Wandel and Goltermann/Narda, Germany). The mobile phone was operated in test mode and was powered through a stabilized power supply, thus antenna power supply was constant. The probe position swept the area corresponding to the rat cage. The measured electromagnetic field values ranged between 26.8 and 40 V/m for the electric field, between 70 and 100 mA/m for the magnetic field and between 0.2 and 0.4 mW/cm^2 for the average power density. These variations can be due to reflections in the Faraday cage. After each birth, mother and its offspring (both males and females) were removed from the Faraday cage and placed inside a separate plexiglas cage.

Plasma and liver samples

After irradiation or sham-exposure to UHF-EMF, animals were killed by decapitation and the blood samples were transferred to a sodium–heparin solution (250 µL heparin/30 mL NaCl 0.9%). Plasma and cell fractions were separated by centrifugation (2000 ×*g*/10 min) and then immediately stored at −70 °C until analysis. The liver was also removed and homogenized (500 mg/2 mL) in ice-cold phosphate saline buffer (PBS, pH 7.4) and stored at −70 °C for further analyses. The results from antioxidant enzyme activities, protein and lipid damage, sulfhydryl content, non-enzymatic antioxidant defense were standardized according to protein content (Lowry et al., 1951).

MN assay

The MN assay was carried out on blood samples collected a moment before decapitation based on previously described guidelines and recommendations (Hayashi et al., 1994; Miller et al., 1997). Briefly, newborn animals two days after birth were randomly chosen from total offspring to perform the MN assays. Pups were individually decapitated and 25 µL of whole blood was smeared on each slides. Two slides were prepared for each animal. Smears were fixed with methanol and air-dried onto slides for 5 min, and cells were stained with Feulgen reagent for 30 min and with Fast Green reagent (0.005% in distilled water) for additional 3 min. The slides were then rinsed with water and air-dried. Neoblasts and micronuclei were morphologically identified and counted in an optical microscope (ZEISS Axiophot, magn, ×100). The slides were coded and scored blindly. A total of 4000 cells were counted to score the number of micronucleated cells per slide and the MN frequencies were normalized for 1000 cells.

Antioxidant enzyme activities

The superoxide dismutase (SOD, E.C. 1.15.1.1) activity in plasma and liver homogenate samples was measured spectrophotometrically by the inhibition rate of auto-catalytic adrenochrome formation in a reaction buffer containing 1 mM adrenaline/50 mM glycine–NaOH (pH 10.2)/1 mM catalase, as previously described (Klamt et al., 2002). Catalase (CAT, E.C. 1.11.1.6) activity was assayed by measuring the hydrogen peroxide (H_2O_2) decreasing rate (Aebi, 1984); erythrocytes and liver homogenate samples (300 µL) were centrifuged (3000 ×*g*; 10 min) and 100 µL of supernatant was mixed with 1 mL of phosphate buffer with 14 mM H_2O_2 (40 µL). Absorbance was followed for 60 s at 240 nm. Glutathione peroxidase (GSH-Px, E.C. 1.11.1.9) activity in plasma and liver homogenate samples was assessed as described by Wendel (1981). NADPH (5 mM) oxidation was followed at 340 nm in the presence of 30 µL of sample, 2 mM reduced glutathione (GSH), mM *tert*-butyl-hydroperoxide, and 0.62 U glutathione reductase in 600 µl of 100 mM potassium phosphate buffer (pH 7.7) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.4 mM sodium azide.

Total sulfhydryl content

The content of sulfhydryl groups in plasma and liver homogenates were determined spectrophotometrically at 412 nm using 5′,5-dithiobis (2-nitrobenzoic acid) (DTNB), which is based on the reaction at neutral and alkaline pH between protein sulfhydryl groups and DTNB, resulting in the formation of the thionitrophenylated protein and a yellow thionitrophenylate anion (Ellman, 1959). Briefly, 100 µl of plasma or liver homogenate were added to a cuvette containing 680 µl PBS (pH 7.4), the blank was set, 20 µl DTNB (2 mM) was added and the absorbance was recorded. Cystein standard curve was made to compare with data from samples.

Proteins carbonyl content

As an index of protein oxidative damage, the carbonyl groups were determined accordingly (Levine et al., 1990). Proteins from 300 µl of plasma or liver homogenate were precipitated by the incubation with thiobarbituric acid (TCA) 20% (100 µl) for 5 min on ice, and then centrifuged at 4000 ×*g* for 5 min. The pellet was dissolved in 100 µl of NaOH 0.2 M, and 100 µl of HCl 2 M or 10 mM of 2,4-dinitrophenylhydrazine (DNPH) in HCl 2 M was added to duplicate aliquots for blanks or for carbonyl groups derivatizing, respectively. Samples were maintained for 30 min at room temperature. Proteins were precipitated with TCA, and washed three times with 500 µl 1:1 ethanol: ethyl acetate with 15 min standing periods to remove excess of DNPH. Samples were dissolved in 200 µl 6 M guanidine in 20 mM KH_2PO_4, pH 2.3 and the absorbance was read at 370 nm. The carbonyl content (nmol/mg protein) was calculated using a molar extinction coefficient of 22,000 M/cm at 370 nm after subtraction of the blank absorbance.

Lipid oxidative damage

Formation of malondialdehyde (MDA), an index of lipidic peroxidation, was assessed by the quantification of thiobarbituric acid-reactive species (TBARS) as previously described

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(Esterbauer and Cheeseman, 1990). Briefly, samples were mixed with 600 μL of TCA 10%, centrifuged (10,000 ×*g*; 10 min) and 500 μL of the supernatant mixed with 500 μL of thiobarbituric acid 0.67% (TBA). Then, the sample was heated in a boiling water bath for 15 min. TBARS were determined by absorbance at 532 nm.

Non-enzymatic antioxidant defense

The total radical-trapping antioxidant parameter (TRAP) assay was performed as previously described (Ghiselli et al., 2000). A sample volume of plasma or liver homogenate equivalent to 100 μg protein was mixed to 4 mL of glycine buffer (pH 8,6) with 2, 2′ azobis (2-amidinopropane) dihydrochloridate (AAPH 10 mM) and 10 μL 5-amino-2,3-dihydro-1,4-phthalazinedione (luminol, 4 mM), and the chemiluminescence produced by the reaction between the peroxyl radicals, formed by the thermal decomposition of AAPH, and the luminol (as external probe for monitoring radical production) was directly proportional to the radicals generated, which were measured in a liquid scintillation counter (Wallac 1409) as counts per minute (CPM).

Statistical analysis

Data were presented as means ± standard error. A computer program (SPSS 8.0, SPSS Inc Chicago IL) was used for statistical analysis. An independent-samples '*t*' test was used to compare the groups. Differences were considered significant at $P<0.05$ level.

Results

MN frequency

This well known assay to detect chromosomal damage induced by toxic agents (Baatout and Derradji, 2004; Silva et al., 2002) was used to analyze the interaction between the UHF-EMF and the genetic material of erythrocyte generator cells. We found a significant increase in erythrocyte MN frequency in newborn pups from irradiated pregnant rats ($P<0.001$, Table 1). The mean of erythrocytes MN frequency scored for each slide analyzed of control or exposed UHF-EMF animals is shown on Table 2.

Oxidative parameters

To better understand the role of free radicals in the UHF-EMF-induced blood chromosome damage and in the liver, we determined total thiol content, protein carbonilation, TBARS, non-enzymatic antioxidant defense and antioxidant enzymes activities. Despite its central role in the regulation of metabolism, the liver was chosen because it is the predominant hematopoietic organ during the second quarter of the fetal phase and an important contributor to hematopoiesis in the newborn (Carlson, 1996).

Protein and lipid oxidative damage

No significant differences between control and UHF-EMF groups were found on carbonyl content of offspring plasma and liver, nor in MDA levels of newborn livers (Table 1). Plasma MDA content was not possible to be determined due to the low concentrations of this compound in newborn blood, which would demand a much higher sample volume to generate reliable absorbance values.

Total sulfhydryl

No significant alterations were found in total thiol content of plasma and liver in the irradiated group (Table 1).

TRAP

We did not detect any differences between the UHF-EMF and control offspring groups in liver homogenate samples (Table 1).

Table 1
Micronucleus frequency and oxidative responses in blood and liver rat offspring after UHF-EMF exposure

Parameter			Control	UHF-EMF
Micronucleus frequency (MN/1000 erythrocytes)	Erythrocytes	$P<0.003$	0.50±0.1 (n=16)	1.23±0.17 (n=27)
Catalase (unit/mg protein)	Erythrocytes	NS	10.41±1.58 (n=18)	11.36±0.92 (n=33)
	Liver	NS	46.03±23.76 (n=18)	46.34±15.63 (n=33)
Superoxide dismutase (unit/mg protein)	Plasma	NS	89.57±9.24 (n=18)	117.27±10.70 (n=33)
	Liver	NS	80.04±7.59 (n=18)	81.24±9.86 (n=33)
Glutathione peroxidase (mmol NADPHoxidado/min/mg proteína)	Plasma	NS	746.72±108.19 (n=10)	626.44±72.00 (n=10)
	Liver	NS	1408.7±49.17 (n=10)	1325.8±41.17 (n=10)
Protein carbonilation (nmol carbonilation/mg protein)	Plasma	NS	5.85±0.96 (n=7)	4.68±0.68 (n=7)
	Liver	NS	5.97±1.23 (n=7)	5.61±1.66 (n=7)
TBARS (MDA nmol/mg protein)	Liver	NS	1.73±0.28 (n=18)	1.69±0.12 (n=33)
Sulphydryl (ng sulphydryl/mg protein)	Plasma	NS	0.44±0.04 (n=18)	0.38±0.04 (n=33)
	Liver	NS	0.20±0.02 (n=18)	0.18±0.01 (n=33)
TRAP (counts per 60 min)	Liver	NS	9263338±545285 (n=10)	8646613±596409 (n=10)
Number of pups per litter		NS	5.75±2.06 [litters (n=4); pups (n=23)]	5.5±1.28 [litters (n=6); pups (n=33)]

Results are expressed as mean±standard error. TBARS = thiobarbituric acid reactive species. MDA = malondialdehyde. TRAP = total radical-trapping antioxidant parameter. The P value is reported for significant variation. NS = not significant.

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Table 2
Erythrocytes MN frequency scored for each slide analyzed of control or exposed UHF-EMF animals

Slide	Control	UHF-EMF
1	0.125	0.625
2	0.250	0.250
3	0.500	1.125
4	1.575	1.000
5	0.825	1.750
6	0.125	0.875
7	0.625	1.375
8	0.625	1.750
9	0.625	0.500
10	0.500	0.375
11	0.375	1.000
12	0.000	3.250
13	0.250	2.175
14	0.250	0.750
15	0.500	0.325
16	0.875	0.125
17	—	0.250
18	—	0.000
19	—	0.500
20	—	1.500
21	—	1.925
22	—	2.000
23	—	1.125
24	—	3.000
25	—	1.750
26	—	1.575
27	—	2.425

The values correspond to the mean of MN scored from two or three independent counts of 1000 cells each from two different slides.
— = no slide.

Enzyme activities

We observed no significant alterations in GSH-Px and SOD activities of offspring plasma (Table 1). The CAT activity in erythrocytes also presented no differences between control and irradiated group (Table 1). We also found no significant differences in liver GSH-Px, SOD and CAT activities (Table 1).

Number of pups per litter

We did not observe a significant difference in the number of pups from UHF-EMF-exposed and control sham-exposed pregnant rats (Table 1).

Discussion

MN frequency

Previously reported works suggest that radiofrequency radiation between 30 MHz and 300 GHz is not directly mutagenic. Despite some conflicting data in the literature, it is often concluded that in vivo and in vitro experiments did not evidence any effect on the induction of micronuclei, sister chromatid exchanges and chromosomal aberrations (Bisht et al., 2002; Brusick et al., 1998; McNamee et al., 2002, 2003; Vijayalaxmi et al., 2001; Zeni et al., 2003). In agreement with our results, other works also reported MN induction in blood cells with different UHF-EMF patterns and exposure times, such as the 837–1909.8 MHz (5.0–10.0 W/kg) UHF-EMF exposure for 3 and 24 h (Tice et al., 2002), the 2.45–7.7 GHz (10–30 mW/cm^2) UHF-EMF exposure for 30 and 60 min (Zotti-Martelli et al., 2000) and the 2.45 GHz [1.5 ± 0.36 (SE) W/kg] exposure for 15 days (Trosic et al., 2004). Results from Reflex Project (REFLEX, 2004) have shown that cells responded to radiofrequency exposure (SAR = 0.3 and 2 W/kg) with a significant increase in single- and double-strand DNA breaks and in MN number. In addition, proteomic analyses on human endothelial cell lines showed that exposure to radiofrequency changed the expression and phosphorylation pattern of large amount of unidentified proteins. Among these proteins is the heat shock protein hsp27, a marker for cellular stress responses. This also can result in DNA damage and cell death (Leszczynski et al., 2004).

According to Goodman and Blank (1998), non-thermal low frequency-EMF induces expression of hsp70. Myc expression was increased by the activation of a 900 bp magnetic field-responsive region in the myc promoter region. The Myc protein induces hsp70 expression by binding to the hsp70 promoter. Hsp70 expression could be then a target to two types of stress-heat and EMF, which operate via different pathways and involve different regions of the same promoter (Blank and Goodman, 2004). However, non-thermal stimulation by either low or high frequency EMF can stimulate the same non-thermal pathway (Blank and Goodman, 2004). Recent works with low frequency EMF also have found increased DNA damage, including enhanced MN formation (Simkó et al., 1999; Wolf et al., 2005; Zmyślony et al., 2000). Wolf et al. (2005) and Zmyślony et al. (2000) suggested that redox reactions are involved in DNA strand breaks.

Protein and lipid oxidative damage

Damage to biomolecules is the main outcome of an oxidative imbalance (Halliwell and Gutteridge, 1999). In contrast with our lipid oxidative damage result another report has shown that markers of lipid damage and kidney tubular damage were increased after UHF-EMF exposure (900 MHz, 1.04 mW/cm^2) for 10 days (30 min/day) (Oktem et al., 2005). Increase in lipid damage was also found in plasma lipid damage in humans exposed for 1, 2 and 4 h to a mobile phone in stand-by mode (900 MHz, SAR not informed) (Moustafa et al., 2001) and in rat brain left hemispheres after UHF-EMF exposure (900 MHz, 2 W/kg) for 7 days (1 h/day) (Ilhan et al., 2004). Differently Irmak et al. (2002) did not find lipid damage in rabbit serum or brain after UHF-EMF exposure (900 MHz, 0.02 mW/cm^2) for 7 days (30 min/day), even when other oxidative parameters – such as SOD activity – were significantly altered. We did not find papers reporting protein oxidative damage in the blood and liver after UHF-EMF irradiation. Our results with MN may suggest that biomolecule damage could be located specifically in DNA.

Total sulfhydryl

The quantification of sulfhydryl groups is an index of total redox status in biological systems. In agreement with our results

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on GSH-Px activity, the total thiol content of plasma and liver was unaltered. Glutathione, an important co-factor of GSH-Px, is part of the total amount of biological thiols quantified by the sulfhydryl assay, and when an imbalance is observed in the thiol pool a decrease in GSH-Px activity is also frequently observed (Halliwell and Gutteridge, 1999). Nevertheless, this result also suggest that there is no significant alterations in the non-enzymatic antioxidant defense in irradiated rats, since thiol-containing molecules are one of the most important component of non-enzymatic defense system in cells.

TRAP

TRAP assay is a well-described method to estimate non-enzymatic antioxidant potential in biological samples subjected to treatments that potentially cause pro-oxidant pulses, since it evaluates the ability of the sample to inhibit AAPH-induced free radicals production. The absence of effects in TRAP assay is in agreement with the previous result on thiol assay and protein and lipid oxidative damage, suggesting that no alterations in free radical production had occurred in the cytoplasm of cells from these tissues. The TRAP assay in plasma also was not possible to be performed in newborn plasma due to the low sample volume from rats at this age.

Enzyme activities

Hook et al. (2004) in a study with cell culture have also found no changes in SOD, CAT and GSH-Px activities after UHF-EMF irradiation (835.62 and 847.74 MHz; 0.8 W/kg) for 20–22 h. Another study using UHF-EMF radiation (900 MHz) and rabbits as an animal model also reported an increase in SOD activity (serum) but it did not show any effect on CAT activity (Irmak et al., 2002). Besides, Moustafa et al. (2001) have found a decrease in plasma SOD activity of humans exposed to a cellular in 'stand-by' for 1 and 4 h, and no effect in CAT activity. The reason for these contradictory data may be due to differences in exposure setups and experimental conditions such as EMF intensity and time exposure to the organisms (Yokus et al., 2005). Also, it is possible that free radical production was enhanced in an intermediary level, which may cause DNA damage, but these levels are not sufficient to induce antioxidant defenses. Cell nucleus generally contain high levels of transition metals, such as iron, that may facilitate DNA oxidative damage through Fenton chemistry, thus producing the highly reactive hydroxyl radical, which is not readily diffusible (Dal-Pizzol et al., 2000; Mello-Filho and Meneghini, 1991). In this regard, the effect of MN formation we observed in this work can possibly be resulting from a localized redox reaction in cell nucleus.

Number of pups per litter

Our results do not agree with the previous works which have related a decrease in offspring number after UHF-EMF exposure to different frequencies, 850 and 2450 MHz and SAR= 1.6 mW/cm^2 (Frazão et al., 2002; Gheyi et al., 2000). Further investigations with larger number of pregnant rats are necessary to clarify this point.

Taken all these data together, we may conclude the examination of oxidative parameters in newborn tissues has not confirmed the hypothesis that free radicals generation is the main mechanism by which UHF-EMF exposure exerts biological effects during interaction with living systems. However, we may not discharge the possibility that pro-oxidant changes are induced in some specific moment during gestation period, or even have placed in another tissue than the ones examined in this work. To address this issue we intend to do more experiments to analyze the hematopoietic tissues oxidant status in different stages of embryogenesis.

There is also a possibility that UHF-EMF effects are detectable only at the nuclear level, such as the chromosome alterations observed in this work. It is well-known that DNA interacts with iron in the nucleus, originating several kinds of DNA–iron complexes (Dal-Pizzol et al., 2000; Mello-Filho and Meneghini, 1991). Some authors suggest that EMF could affect iron homeostasis in biological systems, therefore leading to an increase in cytoplasmic and nuclear free iron, which may enhance hydroxyl radical production via the transition metal-catalyzed Fenton chemistry and cause DNA damage (Lai and Singh, 2004; Stevens, 2004).

Other hypothetic mechanism was proposed to explain a direct effect of EMF exposure in nucleus. Also, some authors suggest that increased electron flux in the DNA molecule during EMF irradiation may induce Hsp70 expression, as cited before (Blank and Goodman, 2004). According to these authors, the energy increase caused by non-thermal EMF irradiation disrupts the weak H-bonds that stabilize the DNA conformation, leading to DNA chain separation and induced stress-response expression.

Evidently, it is not possible to state that the MN frequency increase in irradiated rats would be reproduced in irradiated humans, but the data presented in this work suggests that this is an important issue to be addressed. It is not usual to make phone calls by cell phone for 8:30 h every day, but it is common to carry the device in stand-by close to the body for longer periods. A work has shown that the use of a cellular phone close to the body for few hours increased human oxidative stress parameters (Moustafa et al., 2001). Often adults and children transport the cellular phone close to vital organs and/or hematopoietic tissues, e.g., femur irradiation when the device is placed in the hip. Many pregnant women carry the cellular close to the abdomen and they use the cellular during all the gestation. In an attempt to extrapolate our MN results to human beings we would have taken into account several corrective parameters such as SAR of specific tissues, water content in uterus, size of mother and her pups, number and weight of pups, the width of abdominal wall and the dietary status of the mother and the pups. So these parameters would be calculated in view of an extrapolation.

Considering all the results presented in this work, we conclude that UHF-EMF is indeed able to generate cell chromosome damage transplacentally; nonetheless, whether this damage is dependent or not on an increase in cellular free radicals production remains as an issue to be better understood.

Please cite this article as: Amâncio Romanelli Ferreira et al., Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence..., Life Sciences (2006), doi:10.1016/j.lfs.2006.08.018.

Acknowledgments

We wish to thank the Fundação Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul, and the Conselho Nacional de Desenvolvimento Científico e Tecnológico for financial support.

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