RESEARCH PAPER | Science | Volume : 4 | Issue : 8 | August 2014 | ISSN - 2249-555X

Effects of Chronic exposure to Electromagnetic Waves at 930MHz Frequency on Some Hematological Parameters and cytology of The Bone Marrow of Wistar Rats

KEYWORDS: electromagnetic waves,930MHz, GSM, count, hematocrit, erythrocytes, leukocytes.

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ABSTRACT *Several studies have shown that the electromagnetic waves emitted by mobile phones are suspected to be harmful to health. The effect of chronic exposure of 3 months to electromagnetic waves with a frequency of 930 MHz generated by a wave generator connected to a communication system on hematocrit and blood and bone marrow smears was studied in male and female rats of Wistar strain. 60 rats were divided into 6 groups according to gender (males and females) and the duration of exposure (controls, exposed 1h and 2h).The hematocrit was determined in control and exposed to electromagnetic waves rats and the qualitative study is the recognition of erythrocytes and leukocytes in the blood and bone marrow smears of different groups of animals. The variations in size, shape, color, structure and distribution of these cells have been observed. For quantitative analysis, the blood count was performed using a Malassez cell and the different types of blood cells were observed under a microscope after staining with May-GrünwaldGiemsa. Thestatistical analysiswas performedbyANOVAtestandtheresults were consideredstatistically significantwhen the probabilitypis less than0.05. We observed that the hematocrit and the mean number of erythrocytes increased non-significantly, while the leukocytes decreased non-significantly in rats exposed to electromagnetic waves compared to controls in both sexes and that females are more affected than males. The cytological study of bone marrow and blood smears showed no morphological changes of erythrocytes and leukocytes (monocytes, lymphocytes, neutrophils, eosinophils and basophils) in both sexes. It would seem, according to our results that the exposure of Wistar rats to electromagnetic waves at a frequency of 930 MHz for 3 months did not affect hematological parameters studied,or cytology of the bone marrow.*

Introduction

The exponential increase in the use of mobile phones raises serious concerns about the possible consequences of the interaction between electromagnetic waves and biological systems (Del Vecchio and al., 2009 ;Jajte and al., 2001 ; Lai, and Singh, 1997). These biological consequences, yetlargely unknown, are being studied in laboratory animals (Zhadin, 2001 ;Harakawa and al., 2005 ; Jelenkovic and al., 2005 ; Stefl, and al., 2006) and humans (Feychting, and al., 2005). It is possible to differentiate two broad categories of effects of electromagnetic waves emitted by GSM: measurable thermal effects (International Radiation Protection Assosiation (IRPA), 1988), causing many changes in cells and tissues and non-thermal effects, which occur when the electromagnetic field strength is low enough to increase significantly the temperature of a cell, tissue or or-

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ganism, but some changes can be induced (Cleveland, and Ulcek, 1999).

The hematopoietic system that is a physiological system development is generally more sensitive to noxious stimuli such as microwaves mobile phones.A general blood test based on hematology with the number of erythrocytes, leukocytes and hematocritisan accepted standard for a first indication of a possible biological effect of electromagnetic waves onanimals and humans exposed (Lin, and al., 1979). The results of studies concerning the effects of microwaves on hematological parameters are often contradictory.The reason for these differences is not always easy to identify and may depend on the species used, the duration of exposure and intensity of the electromagnetic waves.

Some authors have reported that electromagnetic waves affect different hematological parameters (Chater, and al., 2006 ;Cakir, and al., 2009). Indeed, these microwaves exerta preponderant influence on hematology of mice andrats (High, and al., 2000 ; Cetin, and al., 2006). A decrease in thenumber of leukocytes was observed in ratsexposed to electromagneticwaves of highintensity (Seto, and al., 1986), but according to these researchers, the effects were subtle. For against, the results of Matausic and colleagues (2000) which exposed rats to electromagnetic waves of 2.45 GHz, 2 hours per day, 5 days per week for 30 days, showed a slight increase in the total number of erythrocytes, with a significant decrease of the leukocytes after 8 days exposure.Furthermore, Ray and Behari (1990) which exposed rats 3 hours per day for 60 days to an electromagnetic field of 7.5 GHz observed an increase in the number of erythrocytes and leukocytes. While Goldoni (1990) observed a statistically significant decrease in leukocytes of workers professionally exposed to electromagnetic waves over a period of two years.It is the same for the work of Budinscak et al (1991), who reported a decrease in erythrocytes and an increase in leukocytes in air traffic employees professionally exposed to microwaves of low intensity over a period of four years. By against, other studies showed no effect of exposure of rats to electromagnetic fields on hematological parameters (Margonato, and al., 1993 ;Vijyalaxmi, and al., 2001).

The aim of our work is to study the effects of chronic exposure of 3 months to 930 MHz electromagnetic fields emitted by a complex system on some hematological parameters (blood count, hematocrit and red blood cell morphology and leukocytes) and cytology of bone marrow of male and female rats control and exposed for 1h and 2h.

Materials and methods

All the procedures mentioned below have been developed in our laboratory : Physiopathology and Molecular Genetics laboratory in Casablanca Ben M'Sik Science Faculty.

Animals

The use of the animals was done in accordance with the manuals of laboratory animals (National Academy of Science of Washington, 1996, Guide for the care and the use of animals laboratory). 60 male and female rats of Wistar strain aged from 29 to 36 days, of the body weight between 60 and 100 g, in good health and belonging to the Faculty of Sciences Ben M'Sik, were used in this experiment. The temperature of the pet store was maintained at 22 ± 2 °C, the humidity around 50 % and the circadian rhythm was 12 h light and 12 h dark. Any observed variation of temperature or illuminance is corrected as soon as possible.

The animals were fed with the standard diet:N° GPF81 of INAAM society, Casablanca, Morocco. The food is distributed daily at the same time. The tap water for drinkingis available ad libitum. The bedding cages are changed three times per week. The rats are identified by a registered number by a permanent marker on the proximal tail part.

The rats were divided into six groups of five rats according to the sex (males and females). The duration of exposure to electromagnetic fields (controls, exposed 1h and 2h) at a frequency of 930 MHz for 3 months on workdays concerned different groups of rats. During the experiment, the rats (5 per group) were housed inside a pyramidal sheet chamber in cages.

Exposure systemto electromagnetic fields

The simulation of environmental of GSM coverage was performed by using a pyramidal structure having a ground base of 1.2 m x 1.2 m and a height of 2 m, the disposal of structure is vertical with a top opening which allows having an omnidirectional antenna allowing a homogeneous radiation of the electromagnetic field.

The radiation was generated by a repeater of 900–1800 MHz that allows reproducing the GSM signal, received from the GSM network and concentrating the radiation inside the test device, which plays the role of a Faraday cage, avoiding the interference with other external signals that can distort the envisaged study. The principle consists to reproduce of an environment similar to the real radiation, while ensuring that is studied only the radiation from GSM network.

Figure 1:The experimental devicefor the production of the signal GSMin the900-1800MHz band(Casablanca BenM'Sik Science Faculty)

We used an amplifier ANDREW (PN: S-2-CPEUS-L-N, LEW POWER SP LIPPER, 800-2500 MHz).

The repeater has two antennas:

- A receiver external antenna which can capture the GSM signal emitted by the nearest GSM station.
- An internal antenna transmitting at a power equivalent to -40 dbm and which can be positioned and oriented according to the desired needs.

The sealing of the device was tested and the signal level was measured by a trace post (Sony Ericsson Phone), with TENS software that performs the measurement in the 2G band 900 MHz. The temperature inside room was measured by a thermometer (digital interior – basic function).

During the exposure to the electromagnetic waves, the

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rats moved freely in a Plexiglas cage of 40 cm× 30 cm ×10 cm. The cage average power densityis about 100 μW/cm^2 (19.4 V/m). In the cell,the cage was placed in the levelwhere an input power could generate the maximum field on the entire surface of the cage. Depending on the cell size, the level of the position of the cage, the input andoutput power, and the SAR (Specific Absorption Rate) were calculated.

Hematological study

Whether it is bone marrow or blood, any puncture implies respect for common rules of antisepsis in order not to contaminate the samples.At the end of the experiment, rats were anesthetized by intraperitoneal injection of 10% ethyluretane, then decapitated by international rules for ethical. Blood samples were collected in tubes containing ethylene diamine tetra-acetic acid (EDTA) and bone marrow was collected from the femur just after decapitation.

- Measurement of hematocrit

The hematocrit was determined in control and exposed to electromagnetic waves rats for 1 and 2 hours by centrifugation of a precise amount of blood (hematocrit tubes calibrated) (HettichHaematokrit D- 7200) and the cell mass/plasma ratio in % was expressed by direct reading on the tube:

H (in %) = (Level of residue) / (overall height) x 100

- Quantitative study of erythrocytes and leukocytes

The quantitative analysis of blood elements consists in a count of these cells by using a Malassez cell. For erythrocytes, the blood collected was diluted 200 times with the liquid Marcano. As for leukocytes, a 20th time dilution was performed in liquid Hayem. The blood cells on the grid were observed by light microscopy with a camera (Leica).

- Qualitative study of erythrocytes and leukocytes

This qualitative study lies in the recognition of erythrocytes and different leukocytes by performing a blood and bone marrow smears in control animals and in 1h and 2h exposed to electromagnetic waves.

A- The blood smear

A blood smear is the blood spread on a microscope slide perfectly clean and degreased to avoid the cell aggregations and the deposition of dyes (Theml, 2000), in order to observe its cells and also count.For its achievement, a gout of blood was spread uniformly on a glass slide, so as to obtain a single cell layer. A second blade is placed on the gout of blood, an angle of 30-45° with the first. The gout was allowed to spread across the width of the second slide by capillary action, then the latter is pulled in the first in a continuous movement, light, without pressing, by an angle of 45°.The smear is then stained with May-GrünwaldGiemsa. The May-GrünwaldGiemsa stained differentially different areas of a blood cell depending on its character acidophil, basophil, neutrophil or eosinophil.The microscopic observation allows the morphological study of cellular blood components, and determining if there is the presence of defects, the appearance or of the cell number.

B- The myelogram

The bone marrow is the site of formation of the precursors of blood cells. The red blood cells, white blood cells and platelets are formed from different cell lines produced in the bone marrow.

The myelogram is the cytological study of the hematopoietic bone marrow. It requires the preparation of a spreading of a juice medullary on a glass slide and its coloring. For the sampling from the bone marrow, different techniques have been described, the most common is that of Endicott and Ott (1945) which is to dissect the femur, immediately after decapitation, and aspirating the bone directly using a needle attached to a syringe, to expel its contents on a slide and realize its spread (Ulich, and Del Castillo, 1991).To facilitate the achievement of the smear, it is advisable to suspend the bone marrow in physiological serum (0.9%). The spread, staining and reading of myelogram are the same as for a blood smear, but by lengthening the time of staining for myelogram, because the cellularity of bone marrow smear is much more important than the blood smear (Valli, and al., 1990).

1- Recognition of erythrocytes

These are the enucleate cells, biconcave disk-shaped flattened in the center and easily recognizable. The variations in size, shape, color, structure and distribution of these cells have been observed.

2- Recognition of leukocytes

The leukocytes are nucleated cells specialized in defending the body against external aggression. They are much less numerous in the blood as the red blood cells. There are several distinct populations of leukocytes (Neutrophils, eosinophils, basophils, granulocytes, monocytes and lymphocytes).

Statistical analysis of results

The results were analyzed using the ANOVA test, which allows comparison of the animals exposed to electromagnetic waves relative to the controls. The results were considered statistically significant if the probability p is less than 5% ($p < 0.05$). The values of the measured parameters were expressed as mean ± standard deviation.

Results

- Measurement of hematocrit and quantitative study of erythrocytes and leukocytes

A non-significant increase ($p > 0.05$) in hematocrit and the mean number of erythrocytes and a non-significant decrease ($p > 0.05$) in the mean number of leukocytes were observed during this experimental study in male and females rats exposed to microwaves relative to the controls (Table 1).

Table 1: Blood counts and hematocrit in control rats and exposed to electromagnetic waves for 1h and 2h

Groups	Sexe	Hematological parameters		
		Hematocrit (%)	Mean number of erythrocytes (10^{12}/l)	Mean numberof leukocytes(10^9/l)
Controls	Males	41,42 ± 1,94	7,66 ± 0,42	8,41 ± 0,64
	Females	40,95 ± 3,6	7,51 ± 0,59	8,35 ± 0,58
Exposed1h	Males	43,94 ± 2,51	7,96 ± 0,6	8,07 ± 0,54
	Females	44,79 ± 4,04	8,04 ± 0,65	7,89 ± 0,62
Exposed2h	Males	48,81 ± 5,61	8,51 ± 0,67	7,68 ± 0,61
	Females	49,82 ± 5,33	8,78 ± 0,69	7,41 ± 0,54

- Qualitative study of erythrocytes and leukocytes

In animals exposed to electromagnetic waves, the quali-

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tative study of erythrocytes revealed no morphological changes, or appearance of erythrocyte inclusions relative to the controls. It is the same for leukocytes, no abnormal granules or nuclei and appearance of cytoplasmic vacuoles and leukocyte inclusions were not observed in exposed to electromagnetic waves relative to the controls (Figures 2 – 13).

A- Male Rats

1- Blood smears

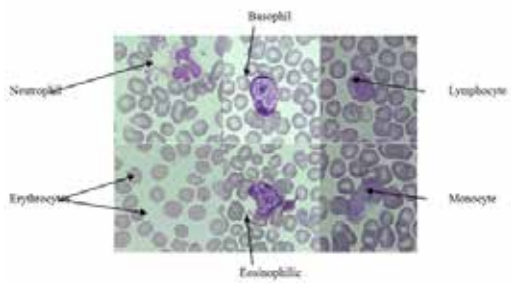

Figure 2: Blood smear from a control rat colored with-May- Grünwald Giemsa(x260)

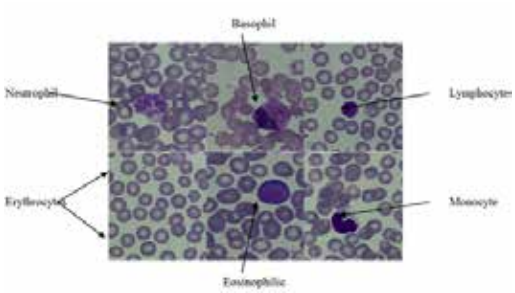

Figure 3: Blood smear from a rat exposed 1h to electromagnetic waves colored with May- Grünwald Giemsa (x260)

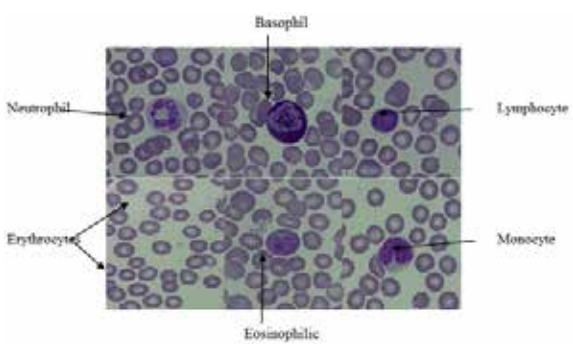

Figure 4: Blood smear from a rat exposed 2h to electromagnetic waves colored with May- Grünwald Giemsa (x260)

2- Myelograms

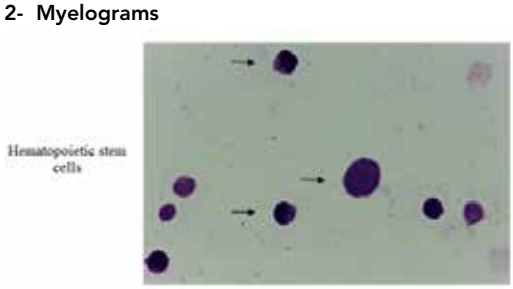

Figure 5: Myelogram from a control rat colored with May- Grünwald Giemsa(x260). The arrows show the hematopoietic stem cells

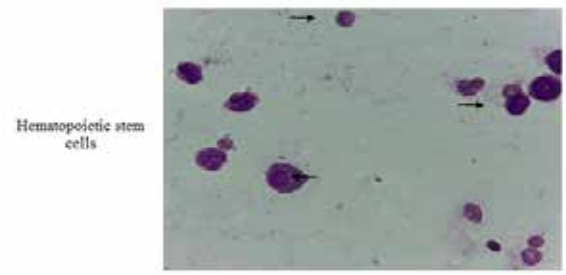

Figure 6: Myelogram from a rat exposed 1h to electromagnetic waves colored with May- Grünwald Giemsa (x260). The arrows show the hematopoietic stem cells

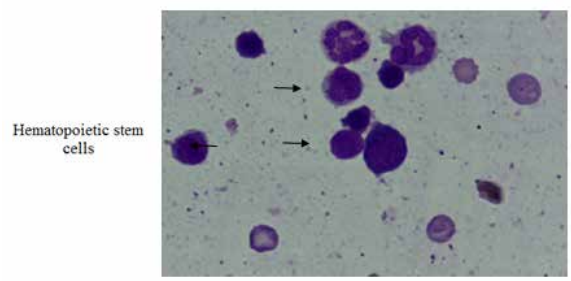

Figure 7:Myelogram from a rat exposed 2h to electromagnetic waves colored with May- Grünwald Giemsa (x260). The arrows show the hematopoietic stem cells

C- Female Rats

1- Blood smears

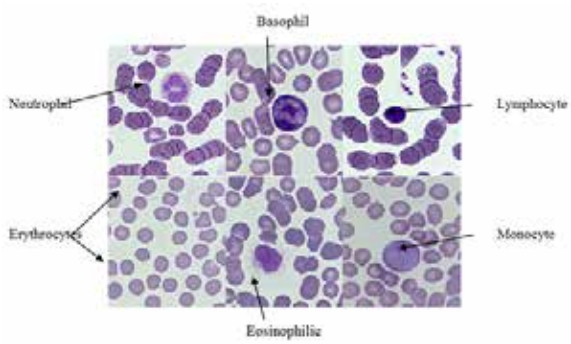

Figure 8: Blood smear from a control rat colored with-May- Grünwald Giemsa(x260)

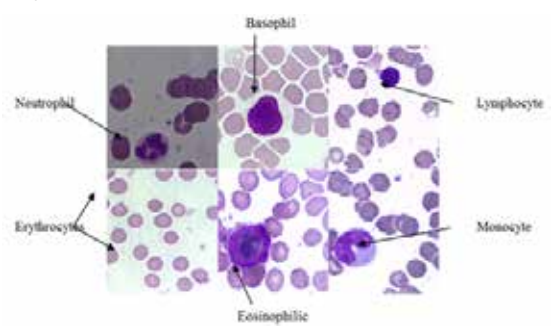

Figure 9: Blood smear from a rat exposed 1h to electromagnetic waves colored with May- Grünwald Giemsa (x260)

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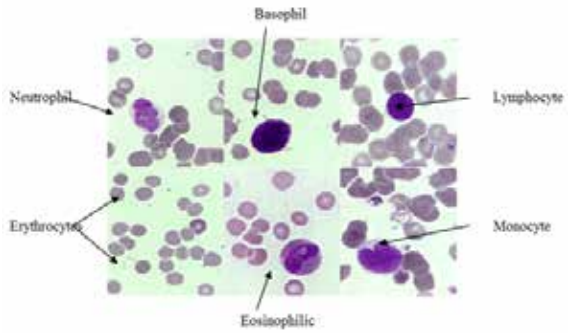

Figure 10: Blood smear from a rat exposed 2h to electromagnetic waves colored with May- Grünwald Giemsa (x260)

2- Myelograms

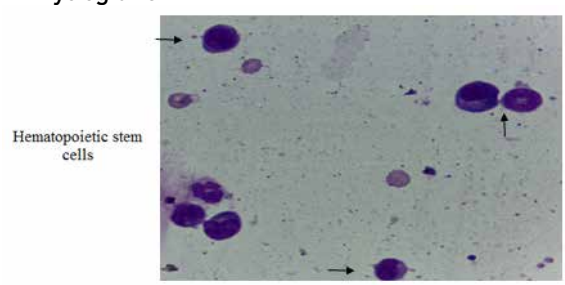

Figure 11:Myelogramfrom a control rat colored with-May- Grünwald Giemsa (x260). The arrows show the hematopoietic stem cells

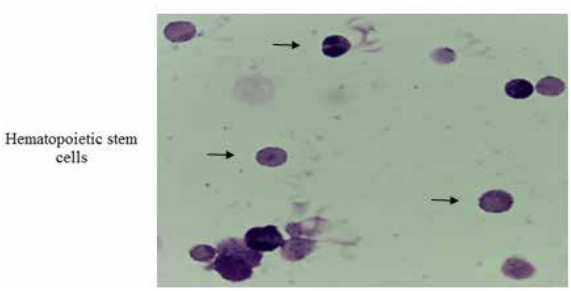

Figure 12:Myelogram from a rat exposed 1h to electromagnetic waves colored with May- Grünwald Giemsa (x260). The arrows show the hematopoietic stem cells

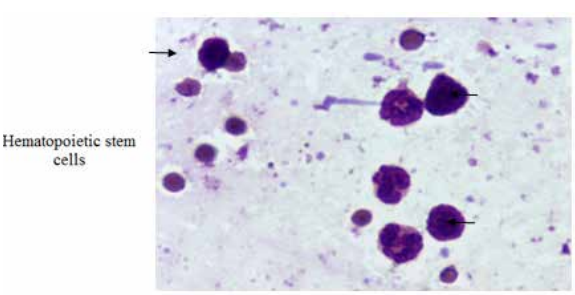

Figure 13:Myelogram from a rat exposed 2h to electromagnetic waves colored with Add space between May-Grünwald and Giemsa (x260). The arrows show the hematopoietic stem cells

Discussion

In this experimental study, the effects of exposure of an hour or two to microwaves at 930 MHz for 3 months on some blood parameters and cytology of the bone marrow were studied in male and females Wistar rats. All the results obtained from the control group were within the normal physiological range (Harkness, and Wagner, 1995).

The hemogram is a quantitative and qualitative study of the various cellular elements of the blood. The quantitative analysis of erythrocytes and leukocytes makes an assessment of the number of cells per unit of volume of the blood and the determination of the hematocrit.

Our work has shown that the values of erythrocytes and hematocrit which represents the relative percentage of the volume of the red blood cells relative to the total blood volume have increased by a non-significantly ($p < 0.05$) after 3 months of exposure in male and female rats. These results can be explained by the installation of a state of hypoxia following exposure to electromagnetic waves (Stashkov, and Gorokhov, 1998). These microwaves depolarize the red blood cells of the body, causing their agglomeration and a slight decrease in the amount of oxygen going to the cells leading to hypoxia (Tomson, 2010). There is a very tight regulation of the amount of oxygen delivered to the tissues and the number of circulating red blood cells. The hypoxia stimulates the secretion of erythropoietin (EPO) by the kidneys, this hormone active the erythropoiesis by causing the proliferation and the differentiation of the erythroblasts in erythrocytes (Fisher, and Nakashima, 1992). Our results are in agreement with those of Adang and Vorst (2006), who exposed the rats to electromagnetic waves of 900 MHz for a period of 21 months. The increase in hematocrit and erythrocytes was also observed by some authors. Indeed, Matausic and al. (2000) also reported an increased number of erythrocytes in the blood of rats exposed to electromagnetic wave (2.45 GHz, 2 hours per day, 30 days). Furthermore, Busljeta and al., (2004) observed an increase in the number of erythrocytes in the blood of male Wistar rats after exposure to electromagnetic waves of 2.45 GHz (2 hours, every day during 15 days) . It is the same for Esfahani and al., (2007) who observed a statistically significant increase in the number of erythrocytes in male rats after exposure to microwaves of 2.45 GHz, thirteen minutes per day for one year.

Other studies have investigated the effects of exposure of rats to electromagnetic waves without significant differences. Indeed, Selmaoui and al., (1996) studied the effect of acute exposure to magnetic fields of 50 Hz on hematological functions in healthy young men. They could not demonstrate any difference between the control and the exposed. Similarly, Margonato and al., (1993) have not shown significant changes in hematological variables in rats exposed to electromagnetic waves of 50 Hz for 1240 hours. Furthermore, Dachà and al., (1993) found no significant change in the number of human erythrocytes exposed in vitro to microwaves.

A physiological adaptation of the exposed to electromagnetic waves to hypoxia may explain the results of these experimental studies (Pequignot, and al., 1997).

For leukocytes, a non-significant increase ($p > 0.05$) of the mean number was observed in our experimental study after 3 months of exposure to electromagnetic waves in male and female rats. Several hypotheses have been advanced to explain these results. The whole body exposure of the rat to radiofrequencies induced thermal stress, which activates the hypothalamic-pituitary-adrenal axis, which triggers the release of corticosteroids in the blood,

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leading to transient changes in blood cell count and other hematopoietic changes (WHO, Environmental Health Criteria 137, 1993). It is possible that due to the prolonged period of exposure of the rats, a cumulative effect could occur. The Hematological variability observed in response to exposure to electromagnetic waves in rats can be explained by overactive spleen (Ragan, and al., 1983) which increases the rate of destruction of leukocytes (Osbakken, and al., 1986). Some studies have shown that microwaves have an effect on hematopoietic stem cells (Bonhomme-Faivre, and al., 1998 ; Cetin, and al., 2006) and their growth factors. The white blood cells appear to be particularly sensitive to the action of the waves acting on the production of interleukins such as IL6, IL3, IL2 and IL4 involved in the maturation of hematopoietic cells.

Our result is in agreement with that reported by Adang and Vorst (2006) who exposed the rats to electromagnetic waves of 900 Mhz. By against, Matausic and al., (2000) who exposed the whole body of male Wistar rats to electromagnetic waves of 2.45 GHz for 30 days (2 hours per day) observed a decrease in the mean number of leukocytes in exposed compared to controls. This decrease in the number of leukocytes was also observed by Svedenstal and al., (1999) in mice exposed for 20 days to microwaves generated by a transmission line of 220 kV. A reduction in the number of leukocytes was also reported by Cetin, and al., (2006) who observed leukopenia after exposure of mice to electromagnetic waves for a period of 120 days.

Other studies have suggested that the electromagnetic fields do not affect the hematological parameters (Margonato, and al., 1993 ;Selmaoui, and al., 1996 ; Dasdag, and al., 2002). Cakir and al., who exposed the rats to a magnetic field of 50 Hz observed no change in mean number of leukocytes (2009). No significant difference was noted with regard to blood parameters and the total number of leukocytes between the controls and the exposed to electromagnetic waves after 63 and 90 days of exposure (Bonhomme-Faivre, and al., 1998).

For the qualitative study of erythrocytes and leukocytes, the observation of blood and bone marrow smears in male and female rats exposed to electromagnetic waves revealed no morphological changes of these structures in rats exposed compared to controls. Thus for erythrocytes, the abnormalities of size (microcytosis and macrocytosis), shape (change of the discoid shape of erythrocytes), and the appearance of erythrocyte inclusions were not observed in this experimental study. As for leukocytes, the abnormalities of granulations and nuclei and the appearance of cytoplasmic vacuoles and inclusions leukocytic were not revealed (Fenneteau, and al., 2006).

As can be seen, the reports on the effects of exposure to microwaves are contradictory. The difference between the results can be attributed to the specificity of the speciesand / or the differences in exposure conditions.

Conclusion

Our results indicate that the exposure to electromagnetic waves can induce some slight non-significant changes in bone marrow and hematological parameters. This variation appears to be within the physiological limits. Nevertheless, our results should be extended and confirmed by other more long-term studies to better understand the effects of electromagnetic waves on the bone marrow and hematological parameters studied.

Due to differences in body size, geometry and physiological responses, the extrapolation of these results to humans is not easy and such comparison must be made with great caution.

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