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Whole body microwave exposure and peripheral blood of rats: pilot study

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ABSTRACT

This study was an attempt to determine biomarkers of non-ionizing, low-level microwave exposure in an animal model. It investigated the effects of continuous-wave radiation on peripheral blood in Wistar rats. The radiation frequency applied was 2450 MHz, while the average power density of the induced field was 10 mW/cm^2. Forty 13-week-old male Wistar rats with an average body mass of 350 g were divided into groups G1 and G2. Group G2 comprised 20 animals, exposed to microwaves for 30 days (5 days a week, 2 hours a day), and group G1 comprised 20 unexposed controls. Peripheral blood samples were taken immediately after irradiation on the 1st, 8th, 16th and 30th day of the experiment. The total number of erythrocytes and leukocytes as well as the differential leukocyte count were investigated and compared. The results revealed an insignificant increase in the total erythrocytes count in microwave-exposed animals ($P>0.05$) in comparison to controls. Total leukocyte count was consistently decreased in irradiated animals from eight day of irradiation ($P<0.05$), while relative lymphocyte count was significantly decreased from the first day. The sight increase in the relative proportion of polymorphonuclear granulocytes was insignificant ($P>0.05$) in comparison to control group. Our preliminary investigation suggests that microwave exposure may affect the haematological parameters of exposed animals, and calls for further evaluation of specificity and biological significance of these biomarkers of exposure.

Key words: microwave irradiation, Wistar rats, non-ionizing radiation, non-thermal effects, haematological parameters

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Introduction

Electromagnetic (EM) radiation is associated with numerous industrial, military, consumer and medical uses. The radiofrequency (RF) portion of EM radiation (spectrum between 0.5 MHz - 100 GHz) seems to affect certain organs and systems (MICHAELSON, 1991). Little is known about health risks from exposure to different sources of non-ionizing radiation. High exposure to non-ionizing radiation acutely affects sensitive organs, which mostly depends on radiation intensity, frequency and exposure duration (GOLDONI, 1994). For the past thirty years much research has been devoted to various aspects of interaction between microwave/radiofrequency (MW/RF) radiation and biological systems. It has been claimed that the conversion of the absorbed energy into kinetic energy is the only significant mechanism involved in such interaction (ANONYMOUS, 1981; MICHAELSON, 1991). The thermoregulatory response in warm-blooded animals, which depends on the coordination of many physiological systems, is well-suited for detection of bio-effects of continuous waves (CW) (FREI et al., 1989). However, discrepancies between empirical observations and theoretical explanations (CLEARY, 1973; DODGE and GLASER, 1977) suggest that non-thermal effects of the RF/MW exposure may also play a role in post-irradiation events, although there is no measurable increase in tissue temperature (ROM, 1983; MCMANUS, 1996; FRANEKIĆ and KOREN, 1997). Non-thermal effects are presently not completely understood (VALBERG, 1997) and require additional investigation.

In order to assess biomarkers of exposure and bio-indicators of radio-effects of low-level microwave exposure, the authors systematically observed experimental animals exposed to CW/MW radiation. This paper is limited to results which refer to MW effects on haematological parameters in the exposed animals.

Materials and methods

Twenty 13-week-old male Wistar rats with an average body mass of 350 g were exposed to continuous microwaves for 30 days, 2 hours a day, 5 days a week. The microwave source was a modified Micro-Chef Moulinex generator with continuous wave operation that produced 2450 MHz. Average power density of the electromagnetic field was 5-15 W/cm^2. The animals were irradiated in Plexiglas cages. Non-irradiated animals matched those exposed in relevant characteristics and served as controls. Both groups of animals were kept at 22±1 °C. During each exposure session, the animals received no feed or water. Both the exposed (G2)

group and control (G1) were divided into four subgroups (N=5 animals per subgroup) and examined for haematological changes on the 1st, 8th, 16th and 30th day of the experiment

To detect a possible increase in body temperature after irradiation we took the rectal temperature of each animal with a thermometer. Blood samples were collected from the tail vein of each animal for haematological analysis. Total erythrocyte and leukocyte counts were determined in a Neubauer chamber using the standard laboratory methods (SOFTIĆ, 1984.)). In order to microscopically estimate the ratio between lymphocytes and granulocytes, blood smears were stained with May-Grünwald and Giemsa standard solution.

Results obtained from irradiated rats were compared to those obtained in controls and evaluated by nonparametric statistical methods (WILLEMSEN, 1974).

Results

Results of haematological analysis showed an increase in the total erythrocyte count in MW-exposed animals (G2-group) (Fig. 1, Table 1) but the difference between the G1 and G2-group was not statistically significant (P>0.05). The shape of the curve seems to be the consequence

Table 1. Total erythrocyte count, leukocyte count and differential cell count in peripheral blood in Wistar rats after microwave irradiation

Day	Treatment[a]	Erythrocyte count (10^9/ml±SD)	Total cell count (10^6/ml±SD)	Differential cell count		
				PMN (%±SD)	Lympho (%±SD)	Others (%±SD)
1st	Control (5)[b]	6.80±0.398	4.12±0.303	14.0±0.7	85.6±0.5	0.4±0.5
	Exposed (5)	7.07±0.418	3.72±0.342	16.4±3.2	81.3±3.3*	2.0±1.4
8th	Control (5)	6.80±0.393	4.22±0.526	16.2±2.2	83.2±2.7	0.6±0.5
	Exposed (5)	7.45±0.943	3.18±0.238*	19.6±3.9	80.2±4.3*	0.2±0.4
16th	Control (5)	6.78±0.525	4.16±0.359	18.4±3.2	80.6±3.3	1.0±0.7
	Exposed (5)	7.38±0.888	2.85±0.539*	20.6±3.5	77.4±4.3*	1.0±1.3
30th	Control (5)	6.92±0.586	4.07±0.204	18.8±1.9	80.0±1.0	1.2±0.8
	Exposed (5)	6.93±0.377	2.39±0.352*	23.4±5.1	76.8±4.4*	1.0±0.7

* significantly different from control, P<0.05 (Mann-Withney test)
[a] microwave irradiation animals (2450 MHz, 10 mW/cm²)
[b] in parentheses: number of animals used

of a wide range of the results, rather than of a statistically marked difference between them. The mean and the median of the total leukocytes count (Fig. 2) decreased with respect to controls (P<0.05), and

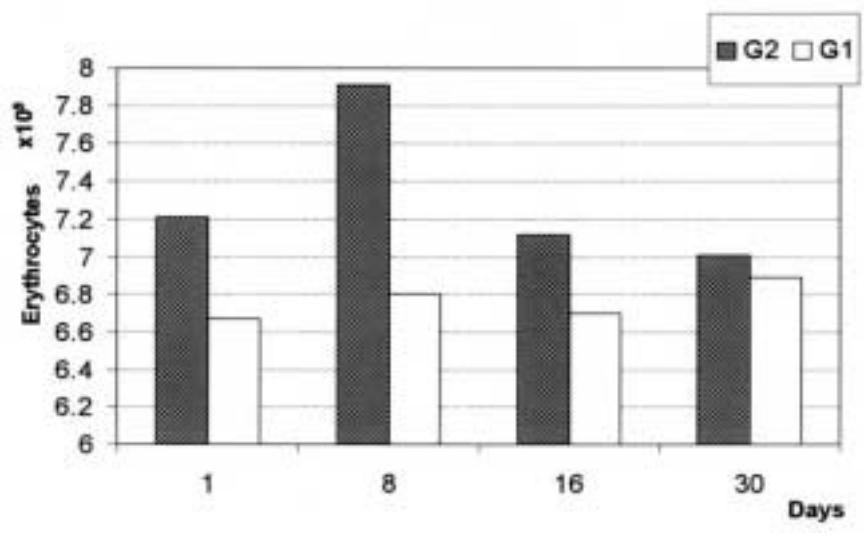

Fig. 1. Total erythrocyte count in peripheral blood after 2450 MHz irradiation in the exposed (G_2) and control group (G_1) of rats

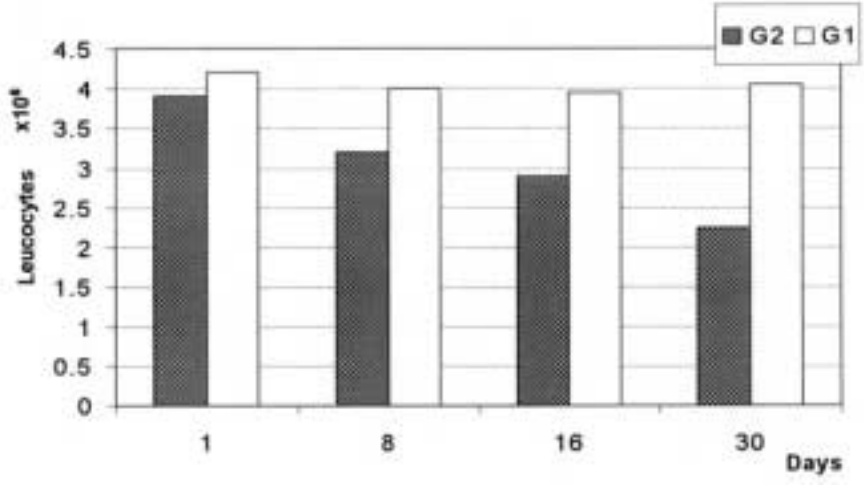

Fig. 2. Total leukocyte count in peripheral blood after 2450 MHz irradiation in the exposed (G_2) and control group (G_1) of rats

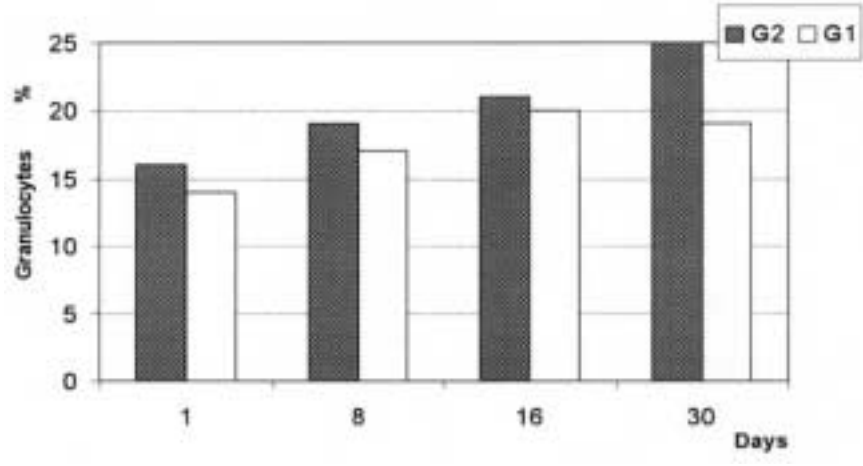

Fig. 3. Percent of granulocytes in peripheral blood after 2450 MHz irradiation in the exposed (G_2) and control group (G_1) of rats

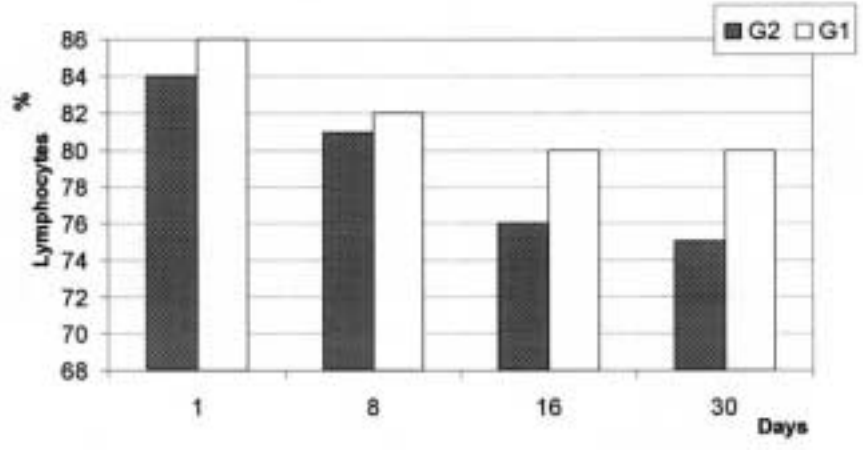

Fig. 4. Percent of lymphocytes in peripheral blood after 2450 MHz irradiation in the exposed (G_2) and control group (G_1) of rats

kept falling from day 8 to day 30. The percentages of lymphocytes (Fig. 4) significantly decreased from day one of the experiment. No significant difference was found in the relative proportion of polymorphonuclear granulocytes between the exposed animals and the control group (Fig. 3).The rectal temperature in experimental animals increased during each irradiation by up to +0.5 °C.

Discussion

Our results show a significant decrease in leukocyte count from day eight onwards, and in lymphocyte percentages from the day first of the experiment. The increase in rectal temperature of exposed animals was insignificant, suggesting that either this level of exposure was too low to produce any thermal effects or that the adaptive potential of the Wistar strain was high enough to compensate for the overheating.

It is difficult to compare the results of our investigation with investigations conducted by others because investigations in the field are often unique. The reasons are the differences between animal species and strains, differences between conditions of exposure, and differences between biological parameters selected for investigation. To our knowledge, there are few authors dealing with the issue of whole body microwave exposure in laboratory conditions.

Most experiments have been conducted in the range of 1-3 GHz (FREI, 1989) but there is no general agreement on non-thermal biophysical mechanisms through which RF electromagnetic fields and MW radiation affect living organisms. The results of numerous studies of haematological effects of microwave radiation are often conflicting. The reason for such discrepancies is not always easy to identify (ANONYMOUS, 1981; CLEARY, 1979; SMIALOWICZ, 1984; LIN et al., 1979) and invites more well-conducted studies on laboratory animals (REPACHOLI, 1997). For example, findings on animal exposure to MW with a power density of 10mW/cm^2-1h per day for 216 days, showed a decrease in total leukocyte and lymphocyte count. However, the proportion of granulocytes increased, whereas other blood parameters remained unchanged (ANONYMOUS, 1981). At the same time, results of the study conducted on rats of three different strains revealed strain-dependency. Namely, regardless exposure duration, after 24000 MHz irradiation two strains of irradiated animals showed significant leukocytosis, lymphocytosis and neutrophilia, while one strain showed the opposite behaviour of the same parameters (SMIALOWICZ, 1984). ĐORĐEVIĆ et al. (1977) found no marked changes in haematological parameters of rats exposed for 90 days (1h per day) to

2400 MHz of continuous microwaves with a power density of 5 mW/cm^2. PAZDEROVA-VEJLUPKOVA and JOSIFKO (1979) exposed young rats to 2736.5 MHz MW-irradiation for 7 weeks at a power density of 24.4 mW/cm^2, and found the number of leukocytes and lymphocytes to be lowered. SMIALOWICZ et al. (1981) found no alterations in haematological parameters in rats chronically exposed to 2450 MHz MW fields. In another experiment, the same author found no consistent change in peripheral blood profile in rats exposed to 425 MHz CW- radiofrequency radiation (SMIALOWICZ et al., 1982).

MCREE (1979) and ROBERTS et al. (1986) reported several haematological variables to be sensitive to RF/MW exposure, not only in animals, but also in humans. Those included white blood cell count, differential white blood cell count, platelet levels, red blood cell count, mitotic index of haematopoietic stem-cells, as well as haematocrit, haemoglobin and bone marrow megakaryocytes. According to these authors, changes in the above parameters repeatedly occurred after either short- or long-term MW/RF exposures, regardless of the power density of the electromagnetic field. In contrast, ROTKOVSKA et al. (1993) found no differences in the total erythrocyte count between irradiated and non-irradiated mice, although she reported a statistically significant decrease in total leukocytes in the exposed animals. Her analyses of blood smears showed a lower percentage of granulocytes in irradiated mice than in controls. The experimental group was exposed to 34 GHz MW radiation with a power density of 20 μW/cm^2. GOLDSMITH (1997) described changes in red and white blood cell count in rats exposed to 13 mW/cm^2 power density MW fields.

Conclusion

The results of this pilot-study of the influence of low-level MW exposure on the haematological status of exposed animals indicated certain changes in the observed parameters, encouraging us to undertake more extensive study of that impact. A long-term investigation is planned to be supplemented by an appropriate *in vitro* experiment that would more precisely determine the specificity and biological significance of radio-effects revealed by this study.

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SAŽETAK

Istraživanje bioloških pokazatelja djelovanja neionizirajućeg zračenja niskih razina izvršeno je na pokusnim štakorima. Istraživan je utjecaj neprekinutih mikrovalova frekvencije od 2450 MHz i srednje gustoće induciranog polja od 10 mW/cm^2 na stanične pokazatelje u perifernoj krvi Wistar štakora. Štakori, 13 tjedana stari, prosjećne tjelesne mase 350 g podijeljeni su u dvije skupine, G1 (N=20) i G2 (N=20). G2 skupina bila je izložena 5 dana u tjednu po 2 sata mikrovalovima, dok je G1 skupina predstavljala kontrolnu skupinu. Pokus je trajao 30 dana. Uzorci krvi uzimani su odmah nakon zračenja 1., 8., 16. i 30. dana pukusa. Određen je i međusobno uspoređen broj crvenih krvnih tjelešaca i ukupni broj bijelih krvnih stanica kao i broj njihovih pojedinih vrsta. Dobiveni rezultati pokazali su da broj crvenih krvnih tjelešaca u mikrovalovima izloženoj skupini nije bio značajno povećan (P>0,05) u odnosu na kontrolnu skupinu. Ukupni broj bijelih krvnih stanica u ozračenoj skupini bio je značajno snižen (P<0,05) od osmog dana, a broj limfocita od prvog dana pokusa, dok je lagano povećanje broja polimorfonuklearnih granulocita bilo statistički neznačajno. Rezultati ovih istraživanja pokazuju mogući učinak mikrovalova na krvne pokazatelje u ozračenih životinja i traže njihovu daljnju potvrdu kao specifičnih biomarkera izloženosti.

Ključne riječi: mikrovalno zračenje, Wistar štakori, neionizirajuće zračenje, netoplinski učinci, krvni pokazatelj