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Frequency of Micronucleated Erythrocytes in Rat Bone Marrow Exposed to 2.45 GHz Radiation

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Abstract

Wistar rats were exposed to 2.45 GHz continuous, radiofrequency microwave (RF/MW) field 2 hours daily, 7 days weekly, at power density 5–10 mW/cm^2. Four subgroups were created in order to be irradiated 4, 16, 30 and 60 hours. Sham-exposed controls were included in the study. Animals were euthanized on the final irradiation day of each treated subgroup. Bone marrow smears were examined to determine the extent of genotoxicity after the particular treatment time. Mann-Whitney test was used for statistical evaluation of data. In comparison to the sham-exposed subgroups, the findings of polychromatic erythrocytes revealed significant differences for the 8th and 15th experimental day. Bone marrow erythrocyte maturation and/or proliferation initiated by subthermogenic RF/MW irradiation showed temporary disturbance. Thereafter, the frequency of micronucleated bone marrow red cells was significantly increased after 15 irradiation treatments. Comparison of micronucleus frequency data obtained after 2, 8 and 30 irradiation treatments did not reveal statistically significant differences between sham and treated subgroups. Under the applied experimental conditions, RF/MW irradiation initiates transitory cytogenetic effect manifested with micronucleus formation in erythropoietic cells.

1. Introduction

Scientific studies performed to date suggest that exposure to radiofrequency fields at intensities far less than levels required to produce measurable heating can cause effects in cell and tissues [1]. Numerous studies regarding RF/MW radiation exposure has resulted in no detectable damage to DNA, or aberrations in chromosomes, or mutation in exposed bacteria, insects, or rodents [2]. In contrast, some authors reported an increase in the number of single- and double-strand DNA breaks in the brain cells, increased incidence of micronuclei formation and specific chromosome aberration in human lymphocytes, and increased micronucleus frequency in rat peripheral erythrocytes after 2.45 GHz irradiation, respectively [3, 4, 5]. So far, studies did not offer clear confirmation about the induction of micronuclei during RF/MW irradiation. *In vitro* obtained results have been reported both positive [6, 7] and negative [8]. It can be presumed that hematopoietic system could reveal microwave effects, even subtle ones, since bone marrow is one of the most proliferative tissues in the body. The aim of this study was to measure the extent of RF/MW genotoxicity by means of micronucleus test in bone marrow immature erythrocytes (PCEs) of rats after 2.45 GHz microwave exposure.

2. Materials and methods

Male Wistar rats (13 week old, approximate body weight 350 g) were used in this study. Both sham-exposed control ($N = 24$) and experimental animal group ($N = 40$) were kept in steady-state micro environment conditions (22°C ± 1°C), and receiving standard laboratory food and water *ad libitum*, with alternating 12-hours light and dark cycles. The experimental group was exposed to 2.45 GHz cw (continuous waves) RF/MW field two hours daily, seven days per week, and every day at the same hour. The experiment lasted thirty days altogether. During the treatment regimen, animals were placed in individual Plexiglas cages and exposed to RF/MW source (modified Micro-Chef Moulinex generator, 900 W, 2.45 GHz) in the far field, at a distance of 1.4 m from the microwave generator. The power density of the field within the individual cages was measured by instrument EM Radiation Monitor, type EMR-20 and 8.2, Wandel & Golterman GmbH & Co. Germany, at "average mode". The results of measurement demonstrated an average power density of the field within 30 seconds. Field power density was selected to be in the range of 5–10 mW/cm^2. Selected power density corresponds to approximate specific absorption rates (SARs) of 1–2 W/kg for middle-sized rat. Mean total body specific absorption rates were estimated according to radiation dosimetry handbook [9]. Calculated SARs of this range exclude thermal stress in rats. Four subgroups of animals were created in order to be irradiated 4, 16, 30 and 60 hours. Sham-exposed animals were included in the study. The body temperature was measured by ThermoScan thermometer (Braun GmbH, Germany) before and after the treatment, to eliminate biased thermal effects on observed variables. Animals were euthanized on the final irradiation day of each treated subgroup. The bone marrow cells were flushed out of femurs [10]. Both the proximal and distal ends of the femur were cut off and the bone marrow cells were gently flushed out with fetal calf serum. The cells were dispersed by gently pipetting and collected by centrifugation at 150 g for 5 min at 4°C. Acridine orange-coated slides were prepared according to Hayashi's method, 1983. Volume of 10 µl of 1 mg/ml acridine orange aqueous solution was spread homogeneously on a warmed glass slide. A volume of 5 µL bone marrow cell suspension was placed on the center of an acridine orange-coated slide and covered immediately with a cover slip. Supravitally stained cells were examined by fluorescence microscope. Immature erythrocytes, i.e. polychromatic erythrocytes (PCEs) were identified by their orange-red color, mature erythrocytes by their green color, and the micronuclei by their yellowish color [11]. For each rat, the number of immature erythrocytes was obtained by examination of 2000 erythrocytes and the frequency of micronucleated polychromatic erythrocytes (MNPCEs) was obtained by observation of 1000 PCEs per slide in randomly chosen fields of vision (magnification ×400). The results were statistically evaluated by using Mann-Whitney test [12].

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Table I. *Incidence of micronucleated polychromatic erythrocytes (PCEs), immature/mature erythrocytes ratio (PCE/NCE) and micronucleus (MN) appearance in the rats' bone marrow after whole-body 2.45 GHz exposure.*

Experimental animal groups	Sacrifice days	Exposure (Hours)	Mean PCEs (PCEs %) ± SD	Mean Ratio PCE/NCE ± SD	Mean MNPCEs ± SD (MN ‰)
Sham-	2	–	59,0 ± 2,9	1,44 ± 0,18	5,3 ± 2,2
exposed control	8	–	57,5 ± 3,1	1,35 ± 0,16	4,8 ± 1,5
animal group	15	–	56,7 ± 3,7	1,31 ± 0,21	4,3 ±1,6
($N = 24$)	30	–	56,0 ± 4,4	1,27 ± 0,24	4,3 ± 1,6
2.45 GHz	2	4	59,0 ± 3,2	1,44 ± 0,19	7,6 ± 2,4
exposed	8	16	63,1 ± 4,7*	1,71 ± 0,34 *	6,6 ± 1,6
animal group	15	30	63,4 ± 4,7*	1,73 ± 0,34 *	9,6 ± 3,3*
($N = 40$)	30	60	60,4 ± 5,6	1,52 ± 0,42	6,5 ± 2,2

*Statistical significant difference $p < 0.05$.

3. Results

Considering applied experimental conditions and preserved body temperature values, changes in the observed parameters throughout of 30-day experiment resulted from athermal effect of microwaves. Descriptive statistic of micronucleated polychromatic erythrocytes incidence, immature/mature erythrocytes ratio (PCE/NCE) and micronucleus appearance in the bone marrow of rats after whole-body 2.45 GHz exposure is given in Table I. Bone marrow cell proliferative activity has been evaluated by inspection of PCE/NCE ratio during the course of experiment. In comparison with sham-exposed controls, analysis of bone marrow from femurs of exposed rats showed a significant increase ($p < 0.05$) in myeloid/erytroid ratio on the 8th and 15th day of experiment, respectively.

Time-course of immature polychromatic erythrocytes in the bone marrow both of rats exposed to 2.45 GHz radiation and sham-exposed controls is shown in Figure 1. Respective significant increase in frequency of PCEs ($p < 0.05$) after cumulative *in vivo* 2.45 GHz exposure of 16 and 30 hours refers that the proliferation and maturation of erythropoietic cells have been affected by irradiation. Figure 2 presents the frequency of micronucleated erythrocytes in the bone marrow of rats exposed to 2.45 GHz radiation and matched sham-exposed controls. Significant increase of micronucleated PCEs ($p < 0.05$) was observed not earlier than experimental day 15, i.e. after 30 hours of cumulative radiation exposure.

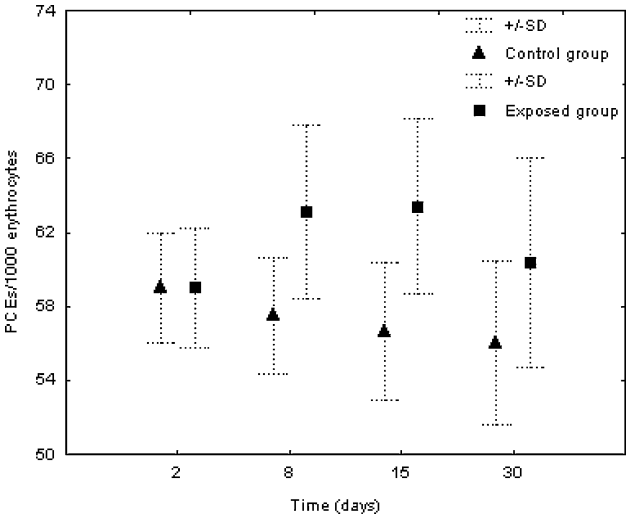

Fig. 1. Time-course of polychromatic erythrocytes (PCEs) in the bone marrow of rats exposed to 2.45 GHz radiation (■) and sham-exposed controls (▲).

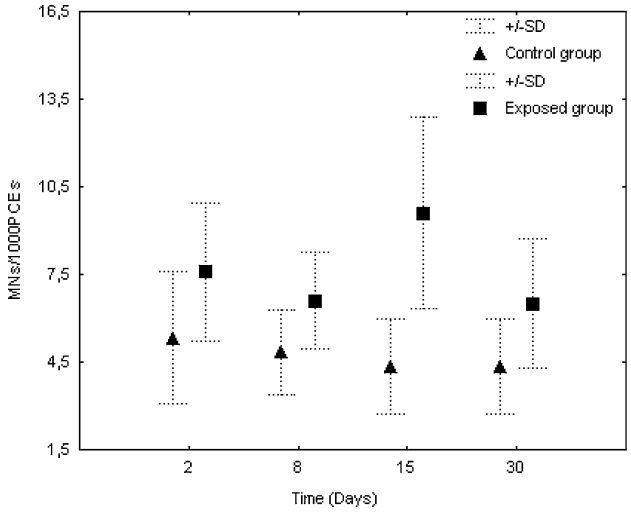

Fig. 2. Time-course of polychromatic erythrocytes (PCEs) in the bone marrow of rats exposed to 2.45 GHz radiation (■) and sham-exposed controls (▲).

4. Discussion

Serious alterations in the rat bone marrow cellularity during 360 hours of exposure to 2.88 GHz have been reported by Ragan [13]. Also, McRee and coworkers indicated an increased myeloid/erythroid ratio in the bone marrow of rabbits exposed to 2.45 GHz cw for 180 experimental days [14]. Recently, Trosic *et al.*, reported leukogram changes, the absolute leukocyte count decrease, and increase of the erythrocyte count in the rat blood after exposure to 2.45 GHz for 30 days [15]. Increased incidence of micronuclei formation in bovine peripheral erythrocytes of cattle accommodated near a radar system [16], as well as increased micronucleus induction after whole-body microwave irradiation in rats [5] has been in contrast to no observed changes in micronucleus frequency in the peripheral blood and the bone marrow of mice chronically exposed to 2.45 GHz for 20 hours a day [17]. The subthermogenic RF/MW field applied in our research has resulted in increase ratio of polychromatic/normochomatic erythrocytes ($p < 0.05$) in exposed animals on experimental day 8th and 15th, respectively. In comparison to the control animal groups, beside the transitory aberration in bone marrow cell proliferative activity, the changes

in cell maturation in irradiated animals has also been found at the same experimental days (Figure 1 and Table I). The increased frequency of micronucleus formation in erythropoietic cells of the rat bone marrow has been observed only after 15 irradiation treatments (Figure 2). Recovery inclination in followed parameters was evident because its values did not significantly differ in comparison with matched controls on the final experimental 30th day. It seems that the mononuclear-phagocyte system (MPS) eliminates altered, i.e., micronucleated, cells and stimulates erythropoietic precursor cells for accelerated maturation by production of hematopoietic growth factors during the sub-chronic microwave exposure [18]. The consequence might be reversible increase of the erythrocyte blood count because of augmented cell inflow from the bone marrow. Macrophages, monocytes, promonocytes, and their precursor cells constitute MPS in the bone marrow. In addition to phagocytosis, it is well-known that these cells also synthesize several cytokines that participate in hematopoiesis (e.g., GM-CSF G-CSF, M-CSF, IL-1, IL-6, IL-12, IFN-α, IFN-β, TNF-α, TGF-β) [19]. These cytokines could trigger a mechanism through which low-level RF/MW radiation affects erythrocytopoiesis. Recent studies that have revealed altered phagocytic and secretory function of peritoneal and alveolar macrophages after RF/MW radiation [20, 21 and 22] strongly support our assumption.

In summary, respective increase in frequency of immature erythrocytes and atypical PCE/NCE ratio on experimental day 8 and 15, as well as observed increase in frequency of micronuclei formation in erythropoietic cells of rat bone marrow only after 15 irradiation treatments indicates an adaptive mechanism to be involved into the proliferation and/or maturation process until the end of experiment.

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