Investigation of the genotoxic effect of microwave irradiation in rat bone marrow cells: in vivo exposure

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An in vivo mammalian cytogenetic test (the erythrocyte micronucleus assay) was used to investigate the extent of genetic damage in bone marrow red cells of rats exposed to radiofrequency/microwave (RF/MW) radiation. Wistar rats ($n = 40$) were exposed to a 2.45 GHz continuous RF/MW field for 2 h daily, 7 days a week, at a power density of 5–10 mW/cm$^2$. The whole body average specific absorption rate (SARs) was calculated to be $1.25 \pm 0.36$ (SE) W/kg. Four subgroups were irradiated for 4, 16, 30 and 60 h. Sham-exposed controls ($n = 24$) were included in the study. The animals of each treated subgroup were killed on the final day of irradiation. Bone marrow smears were examined to determine the extent of genotoxicity after particular treatment times. The results were statistically evaluated using non-parametric Mann–Whitney and Kruskal–Wallis tests. In comparison with the sham-exposed subgroups, the findings of polychromatic erythrocytes (PCE) revealed significant differences ($P < 0.05$) for experimental days 8 and 15. The frequency of micronucleated PCEs was also significantly increased on experimental day 15 ($P < 0.05$). Pair-wise comparison of data obtained after 2, 8 and 30 irradiation treatments did not reveal statistically significant differences between sham-exposed and treated subgroups. Under the applied experimental conditions the findings revealed a transient effect on proliferation and maturation of erythropoietic cells in the rat bone marrow and the sporadic appearance of micronucleated immature bone marrow red cells.

Introduction

There is widespread scientific and public interest in possible health hazards from exposure to electromagnetic fields (EMFs) associated with radiofrequency (RF) and microwave (MW) radiation. This interest has resulted in numerous studies designed to assess both the occupational and residential health risk of EMFs (World Health Organization, 2003). Scientific studies performed to date suggest that exposure to RF fields at intensities far less than the levels required to produce measurable heating can cause effects in cells and tissues. So-called non-thermal effects occur when the intensity of the RF field is sufficiently low that the amount of energy involved would not significantly increase the temperature of a cell, tissue or an organism, yet some physical or biochemical changes are still induced (Cleveland and Ulcek, 1999). Whether or not these low intensity RF-mediated biological effects lead to adverse health effects has not been clearly established. Moreover, without an understanding of mechanisms by which low energy RF fields cause these biological effects, it is not possible to know whether health hazards are associated with exposure to RF sources emitting fields too low to cause a significant temperature rise in tissue.

Most in vivo evidence suggests that RF and MW radiation are not mutagenic and are therefore unlikely to initiate cancers. The majority of studies report lack of a clastogenic effect (Verchaeve and Maes, 1998), but Lai and Singh (1996) reported an increase in the number of single-strand and double-strand DNA breaks in the brain cells of rats exposed to pulsed or continuous wave 2.45 GHz radiation at specific absorption rates (SARs) of 0.6 and 1.2 W/kg. However, a subsequent study did not confirm that DNA damage is produced in cells of the rat cerebral cortex or the hippocampus after a 2 h exposure to 2450 MHz continuous wave (CW) MW or at 4 h after exposure (Malyapa et al., 1998). When mice were exposed to 2.45 GHz at a SAR of 1.18 W/kg for 2 h/day for 120, 150 and 200 days, structural genomic rearrangements were found in brain and testes cells (Sarkar et al., 1994). These studies have been criticized on the basis of deficiencies in the procedures used to process the DNA and the gel electrophoresis methods used to determine the presence of strand breaks (Williams, 1996). Further, a correlation between the frequency of micronuclei and specific chromosome aberration in human lymphocytes exposed to MW of different power densities for different times was reported by Garaj-Vrhovac et al. (1992). Exposure was described as ‘being comparable to everyday environmental conditions’, but no SAR values or temperature measurements were given. The results of the in vitro study by Bish et al. (2002) are inconsistent with the possibility that RF/MW of 835.62 MHz (3.2 or 5.1 W/kg) or 847.74 MHz (3.2 or 4.8 W/kg) induce micronuclei in C3H 10T/2 cells.

Animal models are powerful tools for investigating and understanding the complexity of RF/MW pathogenic potency, since the mechanisms involved are, without doubt, multifactorial. The advantage of such a model is that one can precisely design the experiment, keep the relevant parameters under strict control and imitate the pathological course to be studied, in contrast to the many well-known, mostly ethical limits present during a human study (Trosic et al., 1999, 2000). It is known that activated or developing physiological systems are in general more sensitive to noxious stimuli than static systems. Because erythropoiesis is an ongoing process, there is a continuous progression of cells from erythroblasts to immature and mature erythrocytes, which is balanced in the steady-state condition. As bone marrow is the most proliferative tissue in the body, it could be presumed that the haematopoietic system could reveal MW effects, even subtle ones.

In the present study the possible genotoxicity of non-ionizing radiation was measured by means of the micronucleus

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test in immature bone marrow erythrocytes of rats after sub-chronic 2.45 GHz microwave exposure.

Materials and methods
Male Wistar rats (13 weeks old, body weight ~350 g) were used in this study. A protocol approved by the Animal Care Committee (Institute for Medical Research and Occupational Health, Zagreb) was followed for handling and care of the animals. The animals had passed through a 1 week acclimatization period. Both the sham-exposed control (n = 24) and the experimental animal group (n = 40) were kept under steady-state microenvironmental conditions (22 ± 1°C) and received standard laboratory food and water ad libitum, with alternating 12 h light and dark cycles. The experimental group was exposed to 2.45 GHz CW RF/MW field for 2 h daily, 7 days per week, and every day at the same time. During the treatment regimen animals were placed in individual Plexiglas cages and exposed to a RF/MW source (modified Micro-Chef Moulinex generator, 900 W, 2.45 GHz) in the far field, at the distance of 1.4 m from the MW generator. The individual cages (30 cm high, 8 cm wide, 17 cm long) were designed to house a single rat, thus 40 separated rats, placed in individual cages, were exposed at the same time. The power density of the field within the individual cages was measured with a EM Radiation Monitor, types EMR-20 and 8.2 (Wandel & Golterman GmbH & Co., Germany), set to average mode. Mean total body specific absorption rates were estimated according to a radiation dosimetry handbook (Durney et al., 1980). The whole body average specific absorption rate (SARs) was calculated to be 1.25 ± 0.36 (SE) W/kg. According to the recent literature of an independent expert group on mobile phones, calculated SARs in this range exclude thermal stress in rats (Independent Expert Group on Mobile Phones, 2000). The applied MW power density has been reported by the World Health Organization not to influence body temperature in rats (World Health Organization, 1993). The animals did not receive food and water during exposure sessions. The experiment lasted a total of 30 days. A group of 40 exposed animals was divided into four subgroups which were irradiated for 2 × 2, 8 × 2, 15 × 2 and 30 × 2 h. Rectal body temperature was measured using a ThermoScan thermometer (Braun GmbH, Germany) before and after treatment, to eliminate thermal effects on the observed variables. No significant changes in body temperature were observed in treated animals with respect to controls. On the final day of irradiation of each treated subgroup, the experimental animals were killed under ether anaesthesia immediately after the end of exposure. The control group was also divided into four sham-exposed subgroups to be killed on the indicated days. Both femurs were dissected out from each animal. Bone marrow cells were obtained according to the method of Mazur (1995). Both the proximal and distal ends of the femur were cut off and the bone marrow cells were gently flushed out with foetal 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 method of Hayashi et al. (1983). An aliquot of 10 μl of aqueous acridine orange solution (1 mg/ml) was spread homogeneously on a warmed glass slide. A 5 μl volume of bone marrow cell suspension was placed on the centre of an acridine orange-coated slide. The animals had passed through a 1 week acclimatization period. Both the sham-exposed control (n = 24) and the experimental animal group (n = 40) were kept under steady-state microenvironmental conditions (22 ± 1°C) and received standard laboratory food and water ad libitum, with alternating 12 h light and dark cycles. The experimental group was exposed to 2.45 GHz CW RF/MW field for 2 h daily, 7 days per week, and every day at the same time. 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Supravital stained cells were examined under a fluorescent microscope. Immature erythrocytes, i.e., polychromatic erythrocytes (PCEs), were identified by their orange-red colour, mature erythrocytes by their green colour and micronuclei by their yellowish colour. For each rat, the number of PCEs was obtained from examination of 1000 consecutive PCEs per slide. The results were statistically evaluated using the non-parametric Mann-Whitney and Kruskal-Wallis tests (Willemsen, 1974).

**Table I.** Micronucleus frequencies in the bone marrow of rats exposed to 2.45 GHz radiofrequency radiation

<table>
<thead>
<tr>
<th>Sampling time (days)</th>
<th>Rats per group</th>
<th>Erythrocytes examined per group (×10³)</th>
<th>PCEs/2000 erythrocytes (PCEs %)</th>
<th>MNs/1000 PCEs (MN %)</th>
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*Significant difference P < 0.05.

Results
When we conducted the bone marrow micronucleus test after 2.45 GHz whole body MW irradiation of rats, samples were collected on experimental days 2, 8, 15 and 30, i.e. after 4, 16, 30 and 60 h of cumulative exposure, respectively. The descriptive statistic on PCEs per 2000 erythrocytes and incidence of micronuclei per 1000 immature erythrocytes on the respective irradiation days are presented in Table I.

The results for the time-course of PCEs indicated significant differences (P < 0.05) between control and treated subgroups of animals on experimental days 8 and 15. The PCEs count indicated no significant differences between RF/MW- and sham-exposed animals after 60 h irradiation, i.e. on experimental day 30. The time-courses of PCEs in the bone marrow of non-treated and 2.45 GHz-irradiated animals are shown in Figure 1.

The findings for micronuclei in 1000 immature erythrocytes observed in individual rat exposed to RF/MW radiation ranged from 5.0 to 16.0 on experimental day 15. Otherwise, micronucleus frequency ranged from 2.0 to 6.0 in the match control group on the same experimental day. There was a significant difference (P < 0.05) in micronucleus frequency between sham-irradiated and irradiated groups after 30 h irradiation, on experimental day 15. The time-courses of micronuclei

![Fig. 1. Time-course of polychromatic erythrocytes (PCEs) in the bone marrow of non-treated and 2.45 GHz-treated rats.](https://academic.oup.com/mutage/article-abstract/19/5/361/1240474)
restricted exposure conditions (Brusick radiation are reported to be some subtle indirect effects on controversy. Adverse effects due to exposure of organisms not been confirmed independently and are subject to much fragmentation and on effects on nucleic acid synthesis have mal aberrations did not reveal any effect. Reports of DNA micronuclei, sister chromatid exchange (SCE) and chromosoma- 

Despite inconsistencies in the literature, it is often concluded that radiofrequency radiation in the bone marrow of non-irradiated and 2.45 GHz-treated rats are shown in Figure 2.

**Discussion**

Overviews of the literature suggest that radiofrequency radiation between 30 MHz and 300 GHz is not directly mutagenic. Despite inconsistencies in the literature, it is often concluded that in vivo and in vitro experiments on the induction of micronuclei, sister chromatid exchange (SCE) and chromosomal aberrations did not reveal any effect. Reports of DNA fragmentation and on effects on nucleic acid synthesis have not been confirmed independently and are subject to much controversy. Adverse effects due to exposure of organisms to high frequencies and high power intensities of RF/MW radiation are reported to be some subtle indirect effects on the replication and/or transcription of genes under relatively restricted exposure conditions (Brusick et al., 1998).

Otherwise, an alteration in the length of DNA microsatellite sequences in cells from brain and testis of mice and an increase in the number of single-strand breaks in brain cells of rats has been found. Furthermore, exposure to non-ionizing radiation in the MW range (2.45–7.7 GHz) was able to cause a micronucleus frequency increase in human lymphocytes (Garaj-Vrhovac et al., 1992; Sakar et al., 1994; Lai and Singh, 1995, 1996; Zotti-Martelli et al., 2000). Maes et al. (1996) explored the induction of SCEs in human blood on exposure to 956 MHz for 2 h, at a SAR of 1.5 W/kg. An increase in SCEs in cells exposed to RF irradiation and then treated with mitomycin C has been reported. Chronic in vivo studies by Vijayalaxami et al. (1997) have revealed a significant increase in micronucleus frequency in the bone marrow and peripheral blood of mice exposed to a 2450 MHz signal for 20 h/day, 7 days/week, for 18 months. An increase of 0.05% in MN frequency not correlated with a carcinogenic outcome could not be of biological relevance (Vijayalaxami et al., 1998). Further, in human blood exposed to an 847.74 MHz code division multiple access (CDMA) signal at a SAR of 4.9 or 5.5 W/kg or 835.62 MHz frequency division multiple access (FDMA) at a mean SAR of 4.4 or 5.0 W/kg for 24 h and temperature of 37 ± 0.3°C, respectively, no evidence of chromosome aberration induction due to any applied RF exposure was observed. In an experiment where human lymphocytes were exposed to 2450 MHz pulsed MW for 2 h, at a power density of 5 mW/cm² and SAR of 2.135 W/kg, there was no evidence of induction of DNA single-strand breaks and alkali-labile lesions either immediately or 4 h after exposure (Vijayalaxami et al., 2000, 2001a,b). There was also no evidence for excess genotoxicity in a long-term in vivo study of rats chronically exposed to 1.6 GHz frequency at an intensity of 0.43 mW/cm² and whole body average SAR of 0.036–0.077 W/kg for 2 h/day, 7 days/week over 2 years (Vijayalaxami et al., 2003). Also, no evidence was observed for induction of micronuclei in peripheral blood and bone marrow cells of rats exposed for 24 h to 2450 MHz CW RF at a whole body average SAR of 12 W/kg (Vijayalaxami et al., 2001c). No evidence for induction of genotoxicity in peripheral blood and bone marrow cells of mice exposed to 42.2 GHz, at 31.5 ± 5.0 mW/cm² and SAR of 622 ± 100 W/kg for 30 min/day for 3 consecutive days was found (Vijayalaxami et al., 2004) The aforementioned in vivo and in vitro studies, performed under both chronic and acute conditions and representing a variety of field properties, almost all of them with negative genotoxic outcomes, challenge an interest in intermittent in vivo events in order to assess initial biomarkers of subchronic exposure to RF irradiation.

Our study reports the results of time-course findings on PCEs and incidence of micronuclei in the bone marrow of rats obtained under experimental RF/MW conditions and rectal temperature values. The experiment design has been carefully planned in order to achieve conditions such that thermal effects were avoided. Under the selected and controlled experimental conditions, a maintained rectal temperature and estimated SAR of 1.25 W/kg, we consider that the observed changes derive from non-thermal effects of RF/MW irradiation, although a possibility of localized heating at the selected level of exposure cannot be entirely excluded.

The significant increase in the frequency of immature erythrocytes after 16 and 30 h cumulative in vivo 2.45 GHz MW exposure suggests that the proliferation and maturation of erythrocytic cells were affected by the applied irradiation. The effect became obvious after 8 and 15 irradiation treatments. Erythropoiesis is an ongoing process, i.e. a continuous progression of cells from erythroblasts to immature and mature erythrocytes, which is balanced in the steady-state condition. On the final experimental day the PCE count indicated no significant differences between RF/MW- and sham-exposed animals. The time-course of PCE numbers, after a significant elevation at the beginning, indicates that an adaptive mechanism is involved in the proliferation and/or maturation process until the end of experiment. The results are consistent with findings on the influx of immature erythrocytes into the peripheral circulation after two irradiation treatments previously reported by Trosic et al. (2002). The consequence of an accelerated exit of PCEs from bone marrow is a temporary imbalance in erythrocyte maturation and/or proliferation (Figure 1 and Table I), which activates a known feedback mechanism of the homeostatic control system (Guyton, 2000). A transitory irregularity in cell proliferation, initiated by selected RF/MW irradiation, results in an increase in micronucleus formation in erythropoietic cells of rat bone marrow observed on experimental day 15 (Figure 2). After that, micronucleated PCEs undergo elimination by the mononuclear phagocyte system (MPS), whose functionality is adapted to the higher number of immature and micronucleated cells. Concurrently, the MPS stimulates accelerated maturation of erythropoietic precursor cells by production of haematopoietic growth factors. Macrophages, monocytes, promonocytes and their precursor cells constitute MPS in the bone marrow. In addition to phagocytosis, it is known that these cells also synthesize several cytokines
that participate in haematopoiesis (Unanue, 1993). These cytokines could trigger a mechanism by which low level RF/MW irradiation affects erythrocytosis. Recent studies revealing altered phagocytic and secretory functions of peritoneal and alveolar macrophages after RF/MW irradiation support this assumption (Singh and Bate, 1996; Dasdag et al., 1998; Trosic, 2001). Within the complex net of events observed, including the kinetics of erythropoietic changes at intermittent times during the 30 day experiment, the increase in micronuclei on that particular experimental day remains to be elucidated. The presence of micronuclei may not truly represent a chromosome damaging, i.e. genotoxic or mutagenic, event. Instead it may be evidence of disruption of the mitotic machinery of the cell by the treatment, which will be the subject of our further investigations in this field.

References


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