



**MICRONUCLEUS ASSAY AND LYMPHOCYTE MITOTIC ACTIVITY
IN RISK ASSESSMENT OF OCCUPATIONAL EXPOSURE TO
MICROWAVE RADIATION**

Vera Garaj- Vrhovac

Institute for Medical Research and Occupational Health, Ksaverska c. 2, HR-
10001 Zagreb, Croatia

Corresponding author: Tel.: ++385 1 4673 188; Fax.: ++385 1 4673 303;

E-mail: vgaraj@mimi.imi.hr

(Received in Germany 26 November 1998; accepted 22 March 1999)

Abstract

The effects of radiofrequency electromagnetic radiation (RFR) on the cell kinetics and genome damages in peripheral blood lymphocytes were determined in lymphocytes of 12 subjects occupationally exposed to microwave radiation. Results showed an increase in frequency of micronuclei (MN) as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls. According to previous reports micronucleus assay can serve as a suitable indicator for the assessment of exposure to genotoxic agents (such as RFR) and the analysis of mitotic activity as an additional parameter for the efficient biomonitoring.

©1999 Elsevier Science Ltd. All rights reserved

KEY WORDS: microwave radiation, mitotic activity, micronucleus assay, occupational exposure, human lymphocytes

Introduction

Widely used police and military radars are very potent sources of radiofrequency electromagnetic radiation - RFR (in the non-ionizing range 10 kHz -300 GHz). Biological and possible effects of such exposure have long been a subjects of scientific interest. It is known that exposure to microwave radiation had different biological effects on the eye, the nervous system and its function, circulatory and the reproductive system [1,2,3 and 4]. Results obtained on the level of the cell and results of animal and epidemiological studies proved of great importance in the assessment of potential biological and carcinogenic effects induced by microwave radiation [5,6,7]. Available data on cytogenetic consequences of microwave exposure on the induction of chromosome damage are sometimes contradictory, mostly because of different experimental conditions of *in vitro* and *in vivo* studies [8,9,10 and 11].

The aim of this study was to investigate the effects of microwave radiation on induction and frequency of micronuclei in peripheral blood lymphocytes of occupationally exposed subjects. The mitotic activity of lymphocytes was also studied as an additional parameter for the evaluation of effects in the biomonitoring of microwave radiation exposure.

MATERIALS AND METHODS

Subjects

The study was carried out on 12 clinically healthy workers, nonsmokers, employed on radar equipment and antenna system service. All subjects were male. The subjects worked within a microwave field of $10\mu\text{W}/\text{cm}^2$ -

20mW/cm² with frequency range of 1250-1350 MHz. Their average age was 40.6 yr (21-55 yrs) and employment duration 13.3 yrs (10-19 yrs). The control group consisted of 12 nonsmoking subjects of the similar age. They had never worked with any source of ionizing or nonionizing radiation. For both experimental groups, data were collected on previous diagnostic or therapeutic examinations including exposure to radiation sources or chemical agents.

Methods

Cytogenetic damage in peripheral blood lymphocytes of subjects occupationally exposed to microwave was assessed through micronucleus assay and the analysis of mitotic activity. Both test procedures were carried out on peripheral blood lymphocytes cultivated in *in vitro* conditions.

Micronucleus assay on 72-hours lymphocyte culture was used to measure the risk of occupational exposure to non-ionizing radiation.. Whole blood samples were cultivated *in vitro* in F-10 medium (Gibco) supplemented with fetal bovine serum (Biological Industries, Israel) and phytohaemagglutinin (Murex). After 44 hours of incubation, the lymphocyte culture was added cytochalasin B (Sigma) in final concentration of 3 µg/ml. Preparations were made according to modified method of Fenech and Morley [12]. Lymphocytes were fixed in 3:1 methanol-acetic acid without hypotonic treatment and then stained with Giemsa. For each subject 500 binucleated cells were analysed and the total number and distribution of micronuclei were determined. The significance of differences between the experimental groups was calculated by analysis of variance.

Lymphocyte mitotic activity was determined on slides prepared for sister chromatid exchange (SCE) analysis. Bromodeoxyuridine (BrdUrd) in a final concentration of 10 µg/ml was added to the culture medium. Preparations were

made according to Perry [13]. Cell kinetics was investigated by scoring number of cells in the first (M1), second (M2) and third mitosis (M3). Those cells are easy to distinguish as they differ in the color of chromatides. For each subject 500 cells were analysed. Figure 1 shows results of 72- hours of lymphocyte cultivation for the first, second and third mitosis.

RESULTS

Table 1 shows the frequency of micronuclei and their distribution in binucleated cells for both experimental groups. Maximal number of micronuclei in some subjects of control group was up to 7MN per 500 analysed cells. The majority of binucleated cells contained only one micronucleus, while two subjects had binucleated cells with two micronuclei. The exposed group subjects showed an increase in the number of micronuclei (up to 23MN per 500 analysed cells). The majority of binucleated cells contained 2 or 3 micronuclei. One-way analysis of variance showed significant differences between control and exposed group (F-ratio 17.395).

The cell cycle progression was measured as a percentage of first (M1), second (M2) or third (M3) metaphases following growth of cells in the presence of 5-bromodeoxyuridine(Figure 1).

TABLE 1. Frequency and distribution of micronuclei (MN) in control group and group occupationally exposed to microwave radiation

SUBJECT No	CONTROL GROUP				EXPOSED GROUP			
	No. of binucleated cells with			MN/500 cells	No. of binucleated cells with			MN/500 cells
	1 MN	2 MN	3 MN		1 MN	2 MN	3 MN	
1	5	0	0	5	17	3	0	23
2	4	1	0	6	13	2	0	17
3	2	0	0	2	10	1	0	12
4	5	0	0	5	10	2	0	14
5	7	0	0	7	14	1	0	16
6	3	0	0	3	11	2	0	15
7	5	0	0	5	8	0	0	8
8	2	1	0	4	12	0	0	12
9	2	0	0	2	22	0	0	22
10	2	0	0	2	7	1	0	9
11	3	0	0	3	2	0	2	8
12	6	0	0	6	8	0	0	8

In control group, the 16.6% of cells underwent the first mitosis, 42.7% the second mitosis and 40.6% the third mitosis. Lymphocyte mitotic activity in subjects permanently exposed to microwave radiation indicates disturbance of cell distribution over particularly mitotic cycles. Figure 1 shows the proportion of lymphocytes undergoing the first, the second and the third mitosis for each subject occupationally exposed to microwave radiation compared to control group.

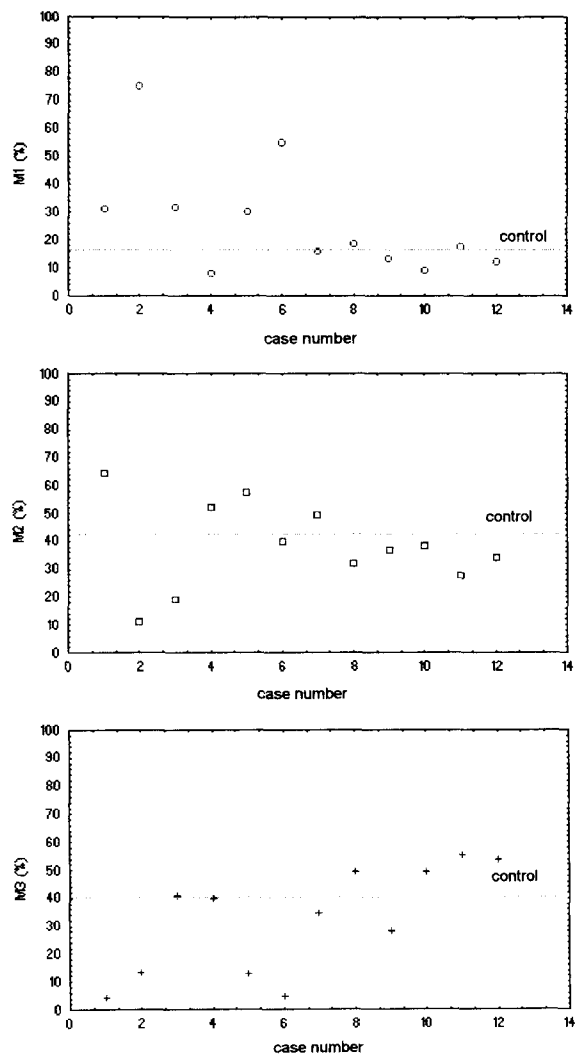


FIGURE 1. Disturbance of cells in the first (M1), second (M2) and third mitosis (M3) of each subject occupationally exposed to microwave radiation compared to control group

DISCUSSION

Since radar equipment is widely used in our environment, it would be important to know whether it can give induce chromosomal alternations such as DNA damage, chromosomal aberrations and/or micronuclei in occupationally exposed subjects. Although possible effects of microwave radiation have received considerable research attention, there is little and different evidence of biological or healthy effects [2,5,7 and 14].

To assess potential genotoxic effects of the non-ionizing radiation, we examined 12 subjects whose everyday work involved exposure to microwave radiation. In order to evaluate continued and recent genotoxic effects we used micronucleus assay and the analysis of lymphocyte mitotic activity on *in vitro* culture of peripheral blood lymphocytes. As a multi-endpoint assay micronucleus test is very useful for simultaneous detection of mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction [15].

The increased number of micronuclei found in the exposed subjects may reflect chromosomal damage or mitotic spindle disturbances during the cell division. Our results confirm previous investigations which indicate that the percentage of chromosome aberrations increases in the lymphocytes of workers occupationally exposed to radar [16]. Similar observations were made by other authors on the cells *in vitro* exposed to radiofrequencies [9,17]. Lai and Singh [11] pointed out that acute exposure to radiofrequency electromagnetic radiation (RFR) at an averaged whole body SAR of 1.2 W/kg causes a significant increase in both single- and double -strand DNA breaks in brain cells of the rats. Moreover, Verschaeve et al. [18] reported an increase in single-strand DNA breaks by alkaline microgel electrophoresis method in lymphocytes from human blood samples exposed to 954 MHz RFR. All these studies point

to chromosomal DNA damages in cells after exposure to RFR. However, some other studies related these damages to the thermal effect and did not show significant mutagenic effect of the RFR exposure [10,19].

Micronucleus assay and the analysis of lymphocyte mitotic activity (study of cell kinetics) are, in addition to conventional cytogenetic assays (structural chromosome aberrations and sister chromatid exchanges), useful screening techniques for determination of recent exposure and evaluation of mutagenic potential of some agents [20,21,22,23].

Present results on mitotic activity and the increased frequency of micronuclei indicate that disturbances in cell distribution over particularly mitotic cycles are more frequent in subjects exposed to microwave radiation than controls (Figure 1). It is possible that, due to non-ionizing radiation, some cells lose their cytokinetic control mechanisms and, stimulated with mitogenic compounds such as phytohaemagglutinin start to divide in their own rhythm of cell division. This may explain evident irregularities observed in cell distribution over mitotic cycles (M1, M2, M3). Previous studies have shown that microwave exposure prevents DNA from entering into the S-phase of cell cycle [8]. These results are in agreement with predictions that proliferative changes resulted from effects of RFR on CHO cells in specific cell cycle phases indicated statistically effects on cells exposed during either G₀/G₁ or S-phase [25].

Direct interaction of the radiation with DNA molecules in *in vitro* conditions result in cell genome damages which persist in the cell for distinct period of time and consequently might cause different malignances [26]. Some epidemiological studies suggest that occupational exposure to electromagnetic fields (EMF) might correlate with disruption in the cells and their functions thus increasing the risk of cancer [7]. It was pointed out that after *in vitro* exposure to microwave radiation the percentage of transformed lymphocytes

(lymphoblastoid forms and macrofages) was twice as higher than in control cultures [27].

The irregularities in cell distribution over the first, the second and the third mitosis together with specific localized structural chromosomal aberrations (especially chromosomal breaks) observed in our study indicate potential risk on lymphatic system disturbances after permanent occupational exposure to microwave radiation [14,28].

The cytogenetic assays might help refine and improve interpretation of cytogenetic data obtained from *in vitro* and *in vivo* studies, that is identification, characterization and quantification of potential cancer risk.

References

1. Adey WR. Tissue interactions with nonionizing electromagnetic fields. *Physiol Rev* 1981; **61**: 435-514.
2. Lin J C. Health aspects of radio and microwave radiation. *J Environ Path Toxicol* 1979; **2**:1413-32.
3. Michaelson SM (1971): Biomedical aspects of microwave exposure. *Am Ind Hyg Assoc J* 1971; **32**: 338-45.
4. Petersen RC. Bioeffects of microwaves. A review of current knowledge. *J Occup Med* 1983; **25**: 103-111.
5. Goldsmith JR Epidemiologic Evidence of Radiofrequency Radiation (Microwave) Effects on Health in Military, Broadcasting and occupational Studies. *Int J Occup Environ Health* 1995; **1(1)**: 47-57.

6. Lai H (1994): Neurological effects of microwave irradiation. In Lin J C (ed): "Advances in Electromagnetic fields in Living Systems." New York: Plenum, 1994; pp 27-80.
7. Szmigielski S. Retrospective analysis of cancer morbidity in Polish military personnel exposed during service to radiofrequency and microwave radiation. In *Abstracts Conference on the Effect of RF EM Radiation on Organisms*, 1994; Skrunda, Latvia.
8. Garaj-Vrhovac V, Koren Z, Horvat Đ. The effect of microwave radiation on the cell genome. *Mutation Research* 1990; **243**: 87-93.
9. Maes A, Verschaeve L, Arroyo A, De Wagter C, Vercruyssen L (1993): In vitro Cytogenic Effects of 2450 MHz waves on Human Peripheral Blood Lymphocytes. *Bioelectromagnetics* 1993; **14**: 495-501.
10. Meltz ML, Walker KA (1987): Radiofrequency (Microwave) Radiation Exposure of Mammalian cells during UV-Induced repair Synthesis. *Radiation Research* 1987; **110**: 255-66.
11. Lai H, Singh NP (1996): Single-and double-strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. *Int J Radiat Biol* 1996; **69(4)**: 513-521.
12. Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. *Mutation Research* 1985; **147**: 129-136.
13. Perry P, Wolff S. New Giemsa method for differential staining of sister chromatids. *Nature (London)* 1974; **251**: 156-158.
14. Garaj-Vrhovac V, Fučić A, Pevalek-Kozlina B. The rate of elimination of chromosomal aberrations after accidental exposure to microwave radiation. *Bioelectrochemistry and Bioelectromagnetics* 1993; **30**: 319-325.

15. Kirschvolders M, Elhajouji A, Cundari E, Vanhummelen P. The in vitro micronucleus test - a multi-endpoint assay to detect simultaneously mitotic delay, apoptosis, chromosome breakage, chromosome loss and non-disjunction. *Mutation Research* 1997; **392**(1-2): 19-30.
16. Garaj-Vrhovac V, Fučić A. Mutagenic response to PHA in lymphocytes cultures from persons occupationally exposed to microwave radiation. In *Abstracts 23rd Annual Meeting of the European Environmental Mutagen Society*, 1993.
17. World Health Organization - WHO (Electromagnetic fields (300Hz to 300 GHz). *Environmental Health Criteria* 1993; **137**:
18. Verschaeve I, Slates D, Van Gorp U, Maes A, Vankerkom J. In vitro and in vivo genetic effects of microwaves from mobile telephone frequencies in human and rat peripheral blood lymphocytes. *Proceedings of COST 244 Meetings on Mobile Communication and Extremely Low Frequency Field: Instrumentation and Measurements in Bioelectromagnetics Research*. 1994; p 74-83.
19. Ciaravino V, Meltz ML, Erwin DN. Absence of a synergistic effect between moderate-power radiofrequency electromagnetic radiation and adriamycin on cell cycle progression and sister chromatid exchange. *Bioelectromagnetics* 1991; **12**: 289-298.
20. Giulotto E, Mottura A, Giorgi R, De Carli L, Nuzzo F. Frequencies of sister-chromatid exchanges in relation to cell kinetics in lymphocyte cultures. *Mutation Research* 1980; **70**: 343-50.
21. Lamberti L, Ponzetto B, Ardito G. Cell kinetics and sister- chromatid-exchange frequency in human lymphocytes. *Mutation Research* 1983;**120**:193-99.

22. Mutchinick O, Ruz L, Gonsebatt M E, Mauleon P, Lisker R, Garcia G (1981): Frequency and types of induced and spontaneous chromosome aberrations in relation to cell kinetics. *Hum Genet* 1981; **59**:137-40.
23. Sasiadek M. Sister-chromatid exchanges and cell-cycle kinetics in the lymphocytes of workers occupationally exposed to a chemical mixture in the tyre industry. *Mutation Research* 1993; **302**:197-200.
24. Garaj-Vrhovac V, Fučić A, Horvat Đ. Comparison of chromosome aberration and micronucleus induction in human lymphocytes after occupational exposure to vinyl chloride monomer and microwave radiation. *Periodicum Biologorum* 1990; **92**: 411-416.
25. Cleary SF, Cao G, Liu LM. Effects of isothermal 2.45 GHz microwave radiation on the mammalian cell cycle: comparison with effects of isothermal 27MHz radiofrequency radiation exposure. *Bioelectrochemistry and Bioelectromagnetics* 1996; **39**: 167-173.
26. Sagripanti JL, Swicord ML. DNA structural changes caused by microwave radiation. *Int J Rad Biol* 1986; **50**: 47-50.
27. Stodolnik-Baranska W. (1967): Lymphoblastoid transformation of lymphocytes in vitro after microwave irradiation. *Nature* 1967; **214**: 102-103.
28. Garaj-Vrhovac V, Hitrec V, Fučić A. Localization of breaks induced by microwave radiation in chromosome of human lymphocytes. In *Abstracts 22nd Meeting European Environmental Mutagen Society*, 1992: