NUCLEIC ACID CHANGES INDUCED BY MICROWAVE AND RADIOFREQUENCY EXPOSURE OF ANIMAL TISSUES

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Abstract. Scientific data reports focused on the physiological perturbations induced by microwaves (MW) and radiofrequency waves (RF) have often referred to professional and accidental exposure to industrial or domestic electromagnetic generators. We aimed to evidence the non-thermal effects of RF and MW acute exposure upon nucleic acids in the animal cells. Animal tissue samples (lung, liver, bone and muscle) have been continuously exposed *in vitro*, for 1–2–4–8–16 hours. Repeated measurements have been carried out in order to ensure the statistical significance of experimental results. The influence of the RF and MW exposure was analyzed and discussed considering the putative non-thermal effects at the level of cell nucleus. The main results concern the slight stimulatory effect of low radiation doses in contrast with the disruptive effect of high dose absorption.

Key words: microwaves, radiofrequency waves, nucleic acids.

INTRODUCTION

The new wireless technology becomes more and more important in human professional and domestic activities. These placed the user in a near field of radiofrequency (RF) electromagnetic waves or microwaves (MW) with an upper level of power density.

One of the most convenient issues is that cancer appearance seems to be not correlated with the electromagnetic irradiation – though various other physiological disturbances are often invoked in carcinogenesis. Various studies have shown that radiofrequency/microwave (RF/MW) radiation and extremely low frequency (ELF) fields cause increased DNA strand breakage and chromosome aberrations. This has been shown in cell lines, human blood, animals and living human beings since the 90th. It means that epidemiological studies in populations exposed to electromagnetic

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radiation (EMR) are likely to show increased cancer, miscarriage and reproductive adverse effects. In fact many epidemiological studies have shown these effects: Goldsmith [4, 5, 6, 7], Szmigielski [13, 14].

In the latest years new investigations have been focused on the biological effects of RF/MW waves related to industrial emissions and electronic communication. Trosic *et al.* [15] showed that in the bone marrow red cells of rats exposed to radiofrequency/microwave, DNA fragmentation during cell division occurs for relatively long exposure times (several hours daily) to a power density of 5–10 mW/cm^2. Also in rats subchronically exposed to RF/MW irradiation at nonthermal level, micronucleated cells' frequency was found increased revealing that RF/MW radiation might cause disturbance in red cell maturation and proliferation, and induce micronucleus formation in erythropoietic cells [2].

Other researchers [8] have examined whether *in vitro* exposure of human peripheral blood lymphocytes to continuous 830 MHz electromagnetic fields causes losses and gains of chromosomes (aneuploidy), a major somatic mutation leading to genomic instability and thereby to cancer; they concluded that the genotoxic effect of the electromagnetic radiation is elicited via a non-thermal pathway. The incidence of DNA fragmentation during cell division (in the form of micronuclei) in the peripheral blood was found significantly increased in the Wistar rats exposed to RF/MW radiation [16] suggesting that an adaptive mechanism, both in erythrocytopoiesis and genotoxicity appeared during the subchronic irradiation treatment. Tice *et al.* [12] demonstrated that, under extended exposure conditions, RF signals at an average SAR of at least 5.0 W/kg are capable of inducing chromosomal damage in human lymphocytes. When human peripheral blood lymphocytes *in vitro* were exposed [17] to electromagnetic fields with different frequencies (2.45 and 7.7 GHz) and power density (10, 20 and 30 mW/cm^2) for three times (15, 30 and 60 min), the cytologic analysis indicated that microwaves are able to cause cytogenetic damage in human lymphocytes mainly for both high power density and long exposure time.

Two plausible biological mechanisms involving free radicals are supposed to be implicated in these effects. The first concerns the increase of free radical activity and genetic damage as a response to exposure. The second involves increased free radical activity and genetic damage because of an induced reduction of a free radical scavenger, e.g. reduced melatonin [11]. It is clear, however, that both mechanisms have the same effect of damaging the DNA and chromosomes.

In the next we aimed to evidence the non-thermal effects of RF/MW electromagnetic acute exposure with respect to nucleic acids molecular damage in the animal cells. The results discussed below are focused on the biochemical changes induced at the level of DNA and RNA acids in freshly excised animal tissues (pork).

MATERIALS AND METHODS

EXPOSURE SYSTEMS

The microwave generator set-up was designed on the basis of an IMPATT diode. It is able to deliver MW flow with the power density of about 10 mW/cm^2 and a frequency of 10.75 GHz. A probe detector measured the power density of electromagnetic wave flux, which was found of about 0.5 mW/cm^2 (low power density microwaves) in the sample plane (at a distance of 25 cm from the horn antenna). The wave reflection has been avoided by using polystyrene bed as tissue sample support. The generator was designed to deliver continuous wave flow all over the duration of the irradiation (Fig. 1). The four tissue samples have been exposed simultaneously for every exposure duration.

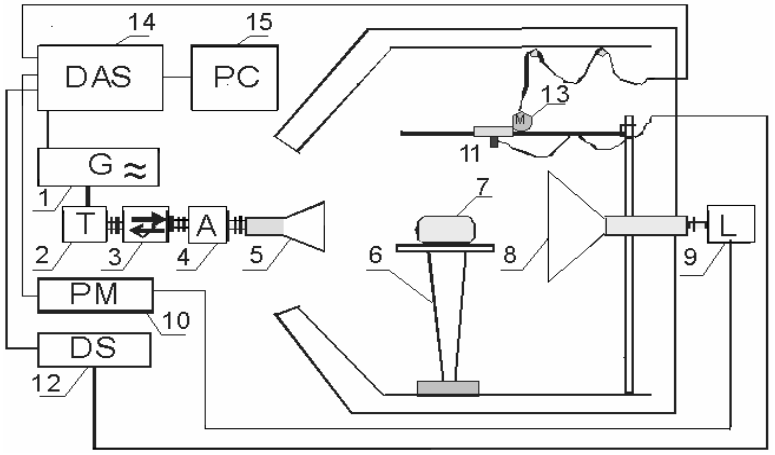

Fig. 1. Microwave exposure system. 1 – microwave power generator; 2 – coaxial coupler; 3 – ferrite isolator; 4 – adapter; 5 – emission horn antenna; 6 – support lossless; 7 – sample; 8 – reception horn antenna; 9 – match load; 10 – power-meter; 11 – probe detector; 12 – voltmeter; 13 – displacement unit; 14 – data acquisition and control system; 15 – computer.

The device used for RF exposure was the transverse electromagnetic cell (TEM) that was designed [3] to deliver 0.6 mW/cm^2 at a frequency of 418 MHz. The TEM device, built in aluminum, has the dimensions calculated to assure the characteristic impedance Z_0 of 50 Ω: a = 715 mm, b = 340 mm, w = 450 mm, where a is the width, b is the height of the rectangular area, w is the septum length (Fig. 2). This way a rectangular coaxial wave guide was obtained that was connected to the power generator through adequate cable so that the transverse propagation mode is the dominant one. When the frequency is tuned on 418 MHz practically the whole electromagnetic energy is propagating along the exposure

device in the form of TEM mode and the power density of the plane wave traveling through the cell can be calculated with:

$$S = \frac{PZ_0}{d^2 Z_\infty} \tag{1}$$

where P – the power supplied by the electric generator, Z_0 the cell characteristic impedance, d – the distance between the central planar conductor and the external one and Z_∞ is the free space impedance.

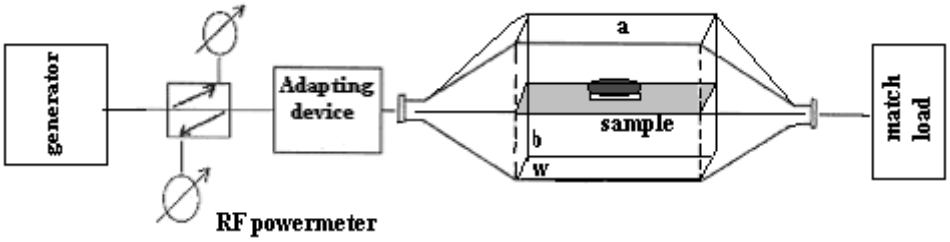

Fig. 2. TEM cell for controlled electromagnetic exposure.

BIOLOGICAL MATERIAL

The experimental research was carried out on fresh pork tissue (liver, lung, muscle and bone). Tissue samples freshly excised from the body and provided by the same individual were cut to reach adequate dimensions for radiation exposure. The samples have been placed in Petri dishes situated in the area with uniform electromagnetic field and energy distribution of the exposure device. For each tissue sample a control one was assayed after keeping it for the same time duration in the same conditions of temperature (about 6 ºC) as the exposed sample, except it was tightly screened against the electromagnetic waves.

The levels of DNA and RNA acids were analyzed in the exposed tissues, five replies of both control and exposed samples being assayed.

NUCLEIC ACID ASSAY

The nucleic acid levels were measured, 24 hours after the exposure was accomplished. Quantities of 0.02–0.03 g cut up from tissues were crushed and submitted to nucleic acid extraction according to standard protocols described in [1]. Spectrophotometric measurements were carried out based on light extinction values in the ultraviolet range at the wavelengths of 270 nm and 290 nm [9, 10], computation of nucleic acid content being performed by means of usual formula:

$$c = \frac{V \cdot (E_{270} - E_{290})}{m \cdot d \cdot 0.19} \tag{2}$$

where: c – concentration of nucleic acids (μg/ g tissue), V – selective solvent extract (ml), E_{270}, E_{290} – light extinction at the wavelength 270 nm, 290 nm, m – tissue mass (g), d – width of spectrometer cells (1 cm), 0.19 – specific extinction for nucleic acids.

Five repetitions of the whole experiment from exposure to spectrophotometric measurement were performed to assure the statistical significance of the experimental data.

STATISTICS

Graphical plots were drawn using average values and standard deviations. The *t*-test was applied to compare the average values of nucleic acid content in exposed samples and control ones.

RESULTS AND DISCUSSION

In figures 3–7 the results of nucleic acid assay were presented for each type of mammal tissue exposed to RF and MW electromagnetic waves. All values corresponding to the exposed samples are given in relative units following raportation to the control values. We mention that for every exposure time a control sample was analyzed – maintained in the same conditions of temperature and humidity till the moment of analysis except the electromagnetic exposure.

In the liver tissue (Fig. 3) the MW exposure resulted in some changes of the nucleic acid average: either supraunitary or subunitary values meaning both increasing and diminution in samples comparatively to the control but they were not correlated with the exposure time.

The exposure to RF waves characterized by about the same power density led to continuous diminishing of the relative values of nucleic acid content, i.e. from 1.25 to 0.60, meaning small increase corresponding to the shortest exposure time (1 hour), approximately the same values as in the control for medium exposure times (2, 4, and 8 hours) followed by considerable diminution (up to 60%) for the longest exposure time (16 hours).

The application of *t*-test showed that most of the differences between the average values obtained for the exposed samples and their corresponding control samples are statistically significant relatively to the significance threshold of 0.05; except the RF wave exposure of 16 hours where the significance threshold of 0.01 was achieved.

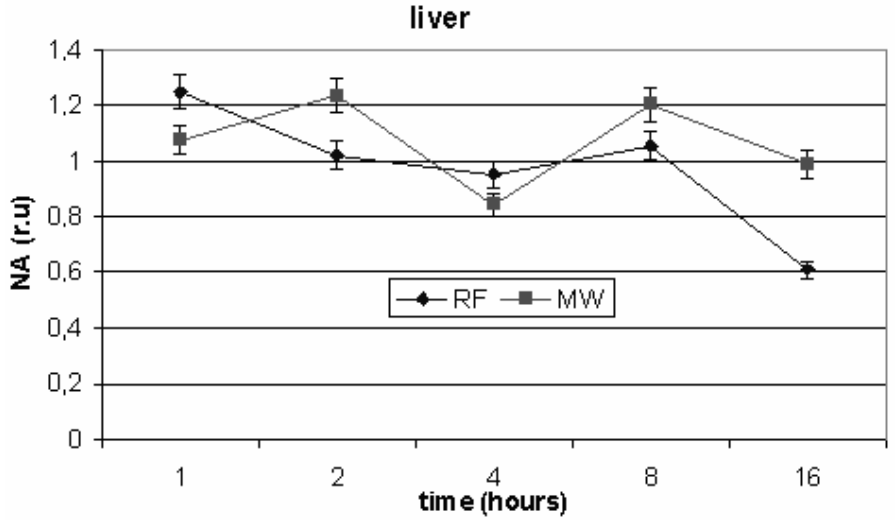

Fig. 3. The response of the liver tissue to MW and RF exposure (r.u. – relative units).

In the lung (Fig. 4) the exposure to MW induced an increase of the average content of nucleic acids from about 1.3 for short and medium exposure times to 2.0 in the sample corresponding to 16 hours exposure time. This is consistent with a slight increase of absolute nucleic acid content in the exposed samples in comparison to the control ones followed by a considerable enhancement for the longest irradiation.

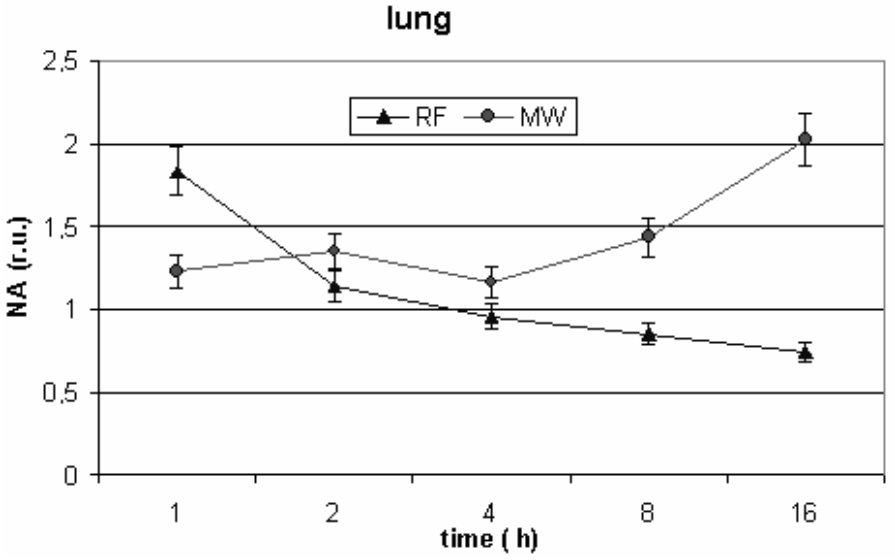

Fig. 4. The changes induced by electromagnetic exposure in the lung tissue (r.u. – relative units).

The condition of statistical significance related to the threshold of 0.05 was satisfied for both MW and RF waves for 1–2–4–8 hours while for 16 hours the statistical threshold of 0.01 was reached.

In bone (Fig. 5) the measurements revealed relative values of nucleic acid content ranging between 0.5 and 0.7 for exposures to MW of 1–2–4–8 hours followed by suddenly raised values to 1.5 for exposure time duration equal to 16 hours. This means that the nucleic acid level was diminished by MW exposure in comparison to the control except for the longest exposure time when the remarkable increase was recorded.

Different response was obtained for RF exposure where subunitary values were recorded only for the longer irradiation.

In the muscle tissue (Fig. 6) both MW and RF exposure induced first the increase of the nucleic acid content in the samples corresponding to 1 and 2 hours exposure times, but approximately non changed values for the exposure time equal to 4 hours. Then, MW exposure resulted also in values over those of the control samples while the RF wave irradiation resulted finally in a considerable diminution for 16 hours exposure time. The possible theoretical hypothesis that could be invoked to shape some explanation for the results obtained in the frame of this experiment could not be related to the thermal effect of electromagnetic exposure since we carried out irradiations at low power density where only non-thermal effects could occur.

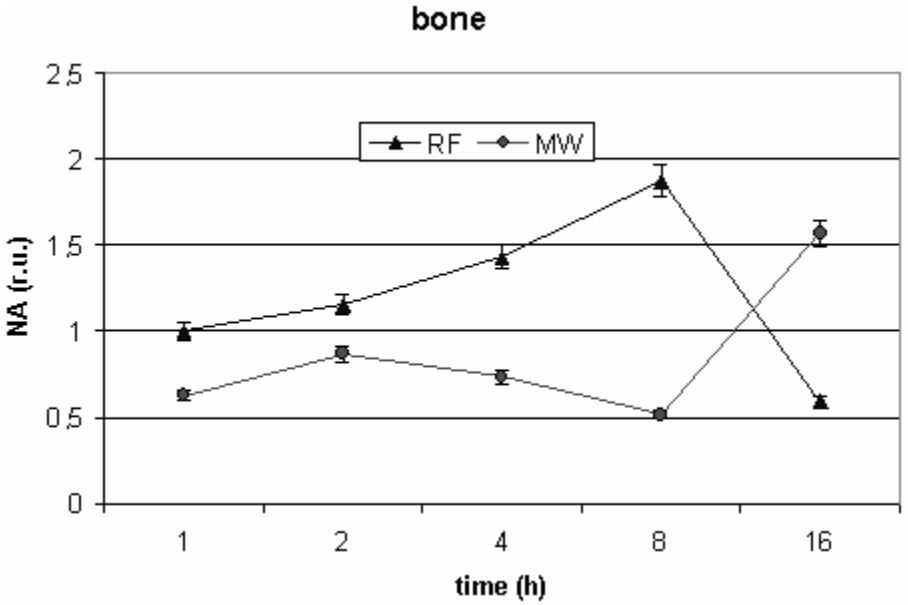

Fig. 5. The influence of the electromagnetic exposure to MW and RF upon the bone tissue (r.u. –relative units).

So, free radicals releasing could be a major cause of damaging nucleic acid molecules or biosynthesis rate could be affected if radiation exposure trigger certain complex biochemical phenomena within the living cell. In the latter case we assume that the exposed tissues still contain living cells though they are studied *in vitro* after extraction from the animal body.

The damaging action of electromagnetic exposure by means of free radicals yielding could be associated with the responses obtained for longer exposure times to RF waves while the biosynthesis rate changes could be considered mainly responsible for the increase revealed for shorter exposure times both for MW and RF irradiation. One might accept that in the case of small amplitude external constraint the cells are able of defence by the intensification of biosynthesis mechanisms so that the damages caused by the cell stressing are surpassed and even superior levels of nucleic acid could result.

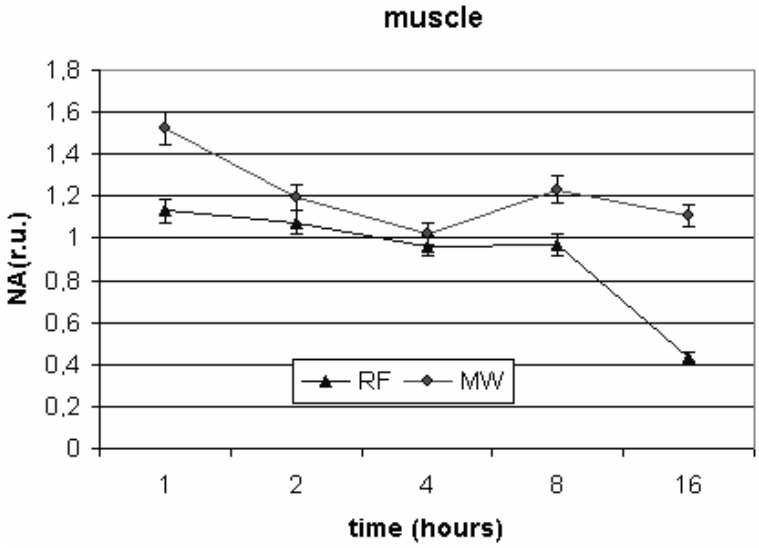

Fig. 6. The changes induced in muscle tissue by the electromagnetic exposure (r.u. – relative units).

We could outline that the most coherent data – representing mostly the increase of nucleic acid levels – correspond to muscle and liver (having a higher content of water) while the decrease of nucleic acid levels in the exposed samples in comparison to the control ones (subunitary relative values) correspond to lung and bone tissues.

CONCLUSION

Electromagnetic exposure was able to induce significant changes in the average content of nucleic acids in spite of low power density, i.e. in the lack of

thermal effects. The most significant modifications were revealed in tissue samples with high water content where the exposure to radiofrequency electromagnetic waves resulted in a slightly stimulatory influence upon the nucleic acid biosynthesis while for short exposure times inhibitory influence was noticed. The genetic risk of professional or accidental electromagnetic exposures needs to be further investigated by using alternative experimental measurement methods.

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