EFFECTS OF MICROWAVE EXPOSURE IN MICE EXPERIMENTAL SUBACUTE TESTS[#]

FELICIA GRĂDINARIU, C. GOICEANU*, R. DĂNULESCU*, DORINA CREANGĂ**, CLAUDIA NĂDEJDE***

* Regional Centre of Public Health Iasi, Romania, e-mail:feligradin@yahoo.com
** Faculty of Physics, „Al. I Cuza" University, Iaşi, Romania

Abstract. The subtle interaction of electromagnetic field (EMF) with the biological matrix might also involve lipidperoxidative processes. Health effects of low-level EMF were studied in a mice short-term experiment. Animals were exposed to an unmodulated ultra high frequency field with a power density of 1 mW/cm^2, producing microwaves (MW) comparable to those emitted by mobile phones. After 15 days of exposure during respectively 1, 2, 4, 8, and 12 hours/day, animals were sacrificed and superoxide dismutase (SOD) activity and thiobarbituric acid reactive substances (TBARS) were assayed in the liver and backlimb muscle. TBARS augmented in both tissues directly with exposure length, excepting the group exposed 12 hours/day where it was drawn towards control level. Muscle SOD activity was inhibited compared to controls, more intensely in the shorter daily exposed animals. In the liver, the higher inhibition rate was observed in the longer exposed groups, probably due to the complex detoxifying and metabolic tasks of this organ. In this experiment, an imbalance in oxidant status consecutive to exposure to EMF could be detected. These changes lead also to some adaptive responses. Further studies are needed, on a greater number of animals and during a longer period of time, to describe the overall effects of MW upon living organisms.

Key words: subacute experiment, microwave, health effects, oxidative stress, SOD, TBARS.

INTRODUCTION

Despite the controversial conclusions concerning health effects of low level electromagnetic field (LLEMF), in 2011, the World Health Organization, International Agency for Research on Cancer in Lyon (IARC) advised that electromagnetic radiofrequency (RF) and microwave (MW) radiation from mobile phone and other wireless devices constitute a "possible human carcinogen" (group 2B) for humans [8]. Another important issue is the impact of RF/MW fields, including cell phone radiation, on various immune functions. Both *in vitro* and *in vivo* studies indicated that, in general, short-term exposure to weak MW radiation

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may temporarily stimulate certain humoral or cellular immune functions, while prolonged irradiation inhibits the same functions [18]. Studies carried out in Sweden have indicated that persons who begin using either cordless or mobile phones regularly before age 20 have greater than a fourfold increased risk of ipsilateral glioma, a specific type of cerebral tumor. High resolution computerized models based on human imaging data suggest that children are more susceptible to the effects of EMF exposure at MW frequencies, probably due to the higher water content in children's tissues and their associated dielectric properties [3]. Data concerning health effects of LLEMF exposure are far to be conclusive. It is rather hard to discriminate these effects because they are subtle and very often they could be confounded by those generated by the normal biological variability [10]. In other cases, these effects could be misdiagnosed as pathological signs of affections generated by a different etiology. There are a lot of experimental studies, either *in vivo* or *in vitro*, which do not provide evidence for any health-threatening effects of LLEMF [5, 13, 19]. There have emerged also studies revealing either adaptive changes to MW exposure *in vitro* [16] or even significant changes like those of protein kinase C activity in a rat experimental model, suggesting that chronic exposures may affect brain growth and development [15]. Although the nonthermal biological effects and mechanisms of LLEMF radiation are still uncertain, the latest data on their mechanisms have indicated that reactive oxygen species (ROS) may play a major role in their biological effects [11]. This was argumented in experimental *in vitro* [17] and *in vivo* [21] studies and even in human healthy volunteers [12].

The lipid peroxidation represents the free radical-induced oxidation of polyunsaturated fatty acids. The intensity of this process can be evaluated by assaying thiobarbituric acid reactive substances (TBARS). Normally, the oxygen free radicals are neutralized by highly efficient systems in the body. These include antioxidant enzymes like superoxide dismutase (SOD). That is why in our experimental study we assessed these markers in the liver and backlimb muscle tissue samples, as effect markers of exposure.

MATERIALS AND METHODS

EXPERIMENTAL PROTOCOL

Swiss white female mice, weighing 30±5g were used, 5/group, housed in individual plexiglas transparent cages, having standard food and water *ad libitum.* Animals were exposed to electromagnetic field respectively 1, 2, 4, 8, and 12 hours daily, for 15 consecutive days. We used a control group of the same age, sex and weight like that exposed, housed in the same room, but never introduced in the exposure device.

Exposure characteristics. Exposure was performed into a transverse electromagnetic cell in an unmodulated field of 1 mW/cm^2 with an operating frequency of 400 MHz. The electric field strength inside the cell was of about 60 V/m and the magnetic field strength of about 0.15 A/m. The characteristics of the exposure device and of the electromagnetic field were described previously [2].

Assay of oxidative stress markers. After 15 days of exposure animals were sacrificed and liver and muscle tissue samples were weighted and frozen at –20^{o}C until assay. TBARS were determined in tissue homogenates in trichloroacetic acid, by the colorimetric method using 1,1,3,3-tetraetoxypropane as standard [20]. SOD activity was determined in saline homogenates, by xanthine oxidase method, with commercial kits provided by Randox Laboratories Ltd. (Antrim UK) [1]. The results were expressed as mmol /g for TBARS and U/g of fresh tissue for SOD.

RESULTS AND DISCUSSION

The mean values ± SD/group of the investigated parameters are presented in Table 1, and the variation of serum TBARS in tissue homogenates is presented in Fig. 1.

Table 1

Mean values ± SD/group for TBARS and SOD in subacute mice exposure

Hours/day of exposure	TBARS (mmol/g fresh tissue)		SOD (U/g fresh tissue)	
	Muscle	Liver	Muscle	Liver
1 hour	12.9±4.9	5.1±1.0	64.2±8.6	49.2±19.2
2 hours	21.8±11.2	5.6±2.6	65.8±21.5	66.8±7.3
4 hours	35.8±18.2	34.1±22.8	81.6±6.1	32.8±2.6
8 hours	35.9±7.1	7.1±2.1	89.5±9.5	38.8±7.2
12 hours	31.7±8.7	5.7±1.1	71.3±5.7	36.6±3.3
Control	24.0±6.7	5.3±1.8	86.5±18.6	77.0±5.3

Muscle TBARS increased with the length of exposure compared to controls in all groups, suggesting that the intensity of lipidperoxidative processes is time-dependent. The highest value, 1.5 times greater than the control, was found in the 4 respectively 8 hours/day exposed mice (t = 2.70, $p < 0.05$). In the 1 h/day exposure group, muscle TBARS were significantly lower compared to control (t = 2.99, $p < 0.05$). In the 12 hours/day exposed group TBARS level was still elevated, exceeding 1.3 folds the control level, but without statistical difference compared to it.

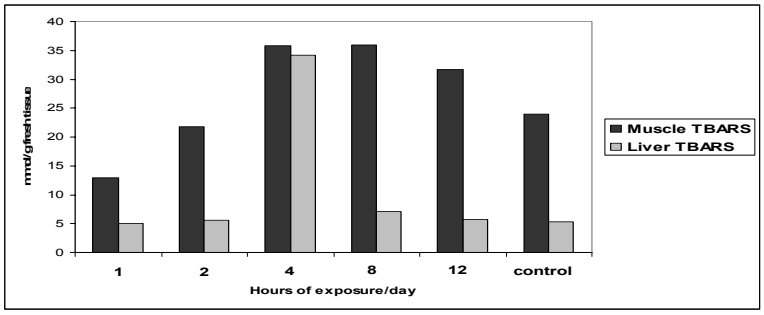

Fig. 1. Liver and muscle TBARS variation in subacute mice microwave exposure.

Liver TBARS varied similarly. In the 4 hours/day group we notice a spectacular increase, 6.4 times the level of unexposed mice. In the longer exposed group TBARS went down to control level. This variation of TBARS in both tissues indicates that after 4 hours of exposure there is a significant enhancement of lipid peroxidation which can be overwhelmed only in muscles in the longer exposed mice, while in the liver it remains at high level of intensity even at a longer duration of exposure.

The profile of ***muscle SOD*** activity, presented in Fig. 2, showed a peak at 2 hours/day exposure, followed by an inhibition in the longer exposed groups, with a tendency of recovery towards the normal values in the longest exposed group, probably due to the activation of some adaptive mechanisms. Anyway this variation of enzyme activity did not reach statistical differences compared to the control group.

The inhibition of ***liver SOD*** (Fig. 2) activity was more intense than in muscle tissue. It remained significantly lower than that of unexposed animals in all 4, 8, and 12 hours/day exposed mice (<0.001). This apparent overwhelming of the antioxidant defense potential might be explained by the multiple and complex metabolical tasks of the liver which involve synthesis, digestion, detoxifying and not in the least antiperoxidative defense.

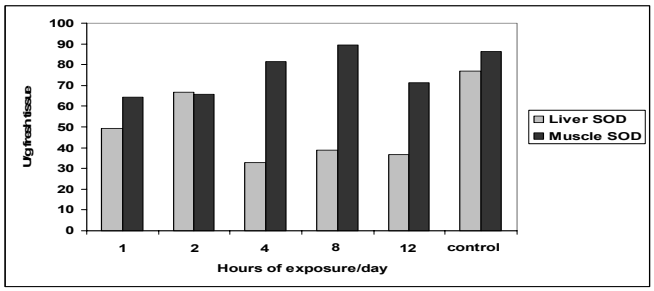

Fig. 2. Liver and muscle SOD activity in subacute mice microwave exposure.

Our results are in line with literature data which have demonstrated similar enhancement of lipid peroxidation in experimental exposure to EMF manifested by augmentation of lipoperoxidative index (malondialdehyde or TBARS) and SOD activity inhibition [7]. Recent studies have found even changes in oxidative balance associated with cytogenetic damage in occupational exposure to pulsed microwave radiation of marine radar equipment [6]. This imbalance in oxidant status was counteracted in some experimental models by treatment with different protecting agents. Melatonin and caffeic acid phenethyl ester, a component of honeybee propolis, were found to be potent free radical scavengers and antioxidants against retinal oxidative stress induced by long-term exposure to 900 MHz electromagnetic field in rats [14]. Bee venom also manifested radioprotective potential *in vitro* against basal and oxidative DNA damage, induced by MW in rat blood lymphocytes [4]. *Ginkgo biloba* extract also prevented the mobile phones-induced oxidative stress in rat brain tissue by preserving antioxidant enzymes (SOD and glutathione peroxidase) activity [9]. These studies confirm that low level of ultra-high frequency EMF might alter the metabolism of free radicals, decrease anti-oxidant capacity and enhance lipid peroxidation.

CONCLUSIONS

In our short term mice exposure to low-level EMF we detected enhancement of oxidative stress, manifested by the increase of tissue TBARS content, more evident in the liver than in the backlimb muscle. Tissue SOD activity was inhibited compared to control, more intensely in the liver than in the muscle, being statistically lower in 4, 8 and 12 hours/day exposed mice. These results refer strictly to our own conditions of exposure. Even if we detected measurable biological changes consecutive to experimental exposure to low level EMF we cannot formulate final conclusions concerning their adverse health effects. Further studies are needed, on a greater number of animals and during longer periods of time. Extrapolation of experimental results to human level is difficult to postulate even on the basis of the many studies gathered in the literature of the last decade. Most recently, *The Bioinitiative Report 2012* [22], a very extended document which integrates the knowledge concerning health effects of EMF exposure, has noted that bioeffects are clearly established and occur at very low levels of exposure to electromagnetic field and radiofrequency radiation. Therefore, the current safety guidelines for EMF exposure are not sufficient and should be revised based on data from various toxicological tests.

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