The Results of Experimental Exposition of Mice by Mobile Telephones

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Abstract – **Today's widespread use of mobile phones has raised the concern about the possible harmful biological effects of long-term exposure to the microwave radiation of low intensity. In vivo investigations of non-thermal influences under the exposure to actual GSM phone radiation comes predominantly from animal studies. In this paper, the results of an experimental exposition of mice by mobile phones are presented. Biological effects of microwave radiation on brain and liver of experimental animals, and increased oxidative stress as a possible pathogenetic mechanism for harmful effects, have been investigated.**

Keywords – **biological effects, mobile phones, exposition, mice**

I. Introduction

The importance of ensuring compatibility between activated electronics instrumentation of various kinds and the pulsed microwave radiation currently used in GSM mobile telephony is well recognised and generally accepted. Prohibition of the use of cellular phones on aircraft and near to the medical devices such as heart pacemakers, hearing aids, defibrillators, etc. is well known because their emissions might adversely interfere with this equipment. Unfortunately, however, the same considerations do not currently extend to the alive human organism, which is generally considered to be immune from adverse influences of GSM radiation, on account of its intensity being far too low to cause any deleterious degree of body tissue heating, as quantified through the so-called specific absorption rate (SAR). It is generally believed that for humans adverse effects can arise only from excessive heating. Indeed, this belief is reflected in the relative leniency of the Safety Guidelines [1] issued by the International Commission for Non-ionising Radiation Protection (ICNIRP). However, the results of many studies suggest that there may be biological effects occurring at low levels of microwave exposure associated with the use of cellular or mobile telephones. The absorbed microwave energy initiates translation, vibration, and rotation of ions and molecules in tissue and these effects should be evaluated for its potential to be hazardous.

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The hypothesis that the low intensity, pulsed microwave radiation currently used in GSM systems can exert subtle, non-thermal influences on the alive organism arises, in the first place, from the fact that the alive human organism itself supports a variety of oscillatory electrical biological activities, each characterised by a particular frequency, some of which happen to be close to those used in mobile telephony.

The particular frequencies used in a GSM system that can be anticipated to be particularly ‘bio-active’ are those of the microwave carrier (900/1800 MHz) and those associated with certain pulsings that characterise the signal employed in the Time Division Multiple Access (TDMA) strategy (the multi-frame repetition rate of 8.34Hz), as well as the 2Hz periodicity associated with the discontinuous transmission mode of the phone. There is evidence [2] that adequately metabolising systems themselves support highly organised, oscillatory electrical activities at the cellular level, whose frequencies generally lie in the microwave band, in terms of which the effects of ultra-low intensity microwaves on fundamental cell processes can be understood in a rather natural way [3]. It should be noted that this endogenous microwave activity is a quite general (non-equilibrium) prediction of modern, non-linear biophysics for living systems, under appropriate metabolic conditions.

Much experimental evidence of non-thermal influences of the microwave radiation on living systems has been published in the scientific literature during the last three decades. Some in vitro studies indicated increased epileptic activity in rat brain as well as impairment of spacing and learning memory in mice as results of increased oxydative stress [4], reduced efficiency of lymphocyte cytoxicity [5], increased permeability of the erythrocyte membrane and increased hemolisation [6], effects on brain electrochemistry (calcium efflux), increase of chromosome aberrations and micronuclei in human blood lymphocytes [1], synergistic effects with cancer promoting drugs such as phorbol ester [7], etc.

In vivo evidence of non-thermal influences, mainly under exposure to actual GSM phone radiation, comes predominantly from animal studies. Some of important findings are: epileptiform activity in rats is enhanced under exposition to the microwave radiation, increase in embryo mortality in rats, increased permeability of the blood-brain and hepatocites membranes in rats, increases in DNA single and double strand breaks in rats, micronuclei formation in liver and brain tissue in rats [8,9].

Human in vivo studies, under GSM or similar conditions, include effects on the human EEG. Influences on the asleep EEG have been reported, including a shortening of rapid eye movement (REM) sleep (with possible adverse effects on learning) during which the power density in the alpha band again increases, and effects on non-REM sleep [10]. Exposure to mobile phone radiation also causes a significant decrease in the preparatory slow potentials in certain regions of the brain and affects memory tasks in human similar that in experimental animals [8,11] .

The aim of this work was investigation of biological effects of microwave radiation on brain and liver of experimental animals and determination of increased oxidative stress as a possible pathogenetic mechanism for harmful effect of long-term mobile phones electromagnetic field exposition.

II. Theoretical Consideration of the Interaction Between Living Matter and EM Field

An attempt to make a theoretical framework for living matter – electromagnetic field (EMF) interactions can be found in [12]. The mechanical work E_{mech} done by EM field, where external electromagnetic wave is incident on system, is [13]

$$\frac{\mathrm{d}E_{mech}}{\mathrm{d}t} = \int_V \vec{J}_{ind}(\vec{r},t)\cdot\vec{E}_0(\vec{r},t)\,\mathrm{d}v \quad (1)$$

where $\vec{J}_{ind}(\vec{r},t)$ is the induced current density in the system and $\vec{E}_0(\vec{r},t)$ denotes the external electrical field.

The induced charge density ρ_{ind} arises by the polarization of the system by the electric fields,

$$\rho_{ind}(\vec{r},t) = -\mathrm{div}\vec{P}(\vec{r},t) \quad (2)$$

where $\vec{P}(\vec{r},t)$ is the polarization of the medium. For the case of a number of molecules with non-overlapping electronic wave function $\vec{P}$ is simply the induced dipole moment per unit volume. In the case of systems where the total charge is conserved (i.e., no charge is added to the system from outside) and where magnetic polarization effects are small, the relation between induced current density and induced charge density satisfies the equation of continuity:

$$\frac{\partial\rho_{ind}(\vec{r},t)}{\partial t} = -\mathrm{div}\,\vec{J}_{ind}(\vec{r},t)\,. \quad (3)$$

Equations (2) and (3) give:

$$\frac{\partial\vec{P}(\vec{r},t)}{\partial t} = \vec{J}_{ind}(\vec{r},t) \quad (4)$$

i.e., the rate of change of the polarization (as defined above) is simply the induced current. Using this relation in (1) we get the alternative expression

$$\frac{\mathrm{d}E_{mech}}{\mathrm{d}t} = \int_V \frac{\partial\vec{P}(\vec{r},t)}{\partial t}\cdot\vec{E}_0(\vec{r},t)\,\mathrm{d}v \quad (5)$$

for the mechanical work done by the external field on the system.

By assuming a harmonic time-dependence of the external electrical field

$$\vec{E}_0(t) = \mathrm{Re}\left[\vec{E}_0(\vec{r})e^{-jwt}\right] = \frac{1}{2}\left[\vec{E}_0(\vec{r})e^{-jwt} + \vec{E}_0(\vec{r})^* e^{-jwt}\right],$$

and the same for $\vec{P}(\vec{r},t)$, the time-averaged power of (5) becomes

$$\left\langle\frac{\mathrm{d}E_{mech}}{\mathrm{d}t}\right\rangle = \frac{1}{2}\mathrm{Re}\left[-jw\int_V \vec{P}(\vec{r})\cdot\vec{E}_0(\vec{r})\,\mathrm{d}v\right] = \frac{w}{2}\mathrm{Im}\left[\int_V \vec{P}(\vec{r})\cdot\vec{E}_0(\vec{r})^*\,\mathrm{d}v\right] \quad (6)$$

The mechanical energy can either be absorbed by the system or reradiated to the surroundings (scattering). It is customary to define the extinction (or total) cross section, σ, according to

$$\sigma = \frac{(\text{Absorbed energy+scattered energy})/\text{unit time}}{\text{Energy flux of the incoming radiation}} = \sigma_{abs} + \sigma_{scatt}$$

The energy flux of the incoming radiation is $c\varepsilon_0\left|\vec{E}_0\right|^2/2$. The extinction cross section for a harmonically time-varying field then takes the form

$$\sigma(\omega) = \frac{k}{\varepsilon_0\left|\vec{E}_0\right|^2}\mathrm{Im}\left[\int_V \vec{P}(\vec{r})\cdot\vec{E}_0(\vec{r})^*\,\mathrm{d}v\right] \quad (7)$$

Here, $k = \left|\vec{k}\right| = \omega/c = 2\pi/\lambda$, where $\vec{k}$ is the propagation vector of the electric field.

The extinction is a measurable quantity and the obtained result can be used to get the cross section for molecules and molecular aggregates. Further, some dielectric functions can be defined and electromagnetic response of electrolytes can be found in this way [12]. This theoretical analysis helps in better understanding the behavior of the cells in living matter when they are exposed to EM field.

Some other theoretical considerations of the electromagnetic response of living matter can be find in scientific literature, for instance [2], [14], etc.

III. Results of the Experiment

In this experiment we used BALB/c mice from the same clutch. The mice were 3 weeks old at the beginning of experiment and they were divided into two groups: I-experimental group, consist of 4 female and 4 male animals, II-control group, consist of 8 female and 4 male animals.

All experimental animals were suited in the same room with daylight and without near sources of microwave radiation, in the cages 30x40x40cm (WxLxH). Experimental group was continually exposed to the microwave radiation from mobile phones (GSM, 900MHz frequency band). In adding to stand-by exposure, we simulated 30 minutes of telephone using every-day at the different time. The source of microwave radiation (a mobile telephone) was situated in the center of cage, while the distance of the source from the floor was 3 cm and maximal distance from the floor corners was 28.2 cm. Amount of the food was not limited and animals were fed *ad libidum*. Experimental group was exposed to microwave radiation during 2 months.

Biological effects were determined by observation of individual and collective behavior and body mass changes. Histopathologic examination was done on paraffine sections from liver and brain, stained with HE (haematoxyline eosin) Lipid peroxidation was determined by measuring quantity of malondialdehyde (MDA) concentration (secondary product of lipid peroxydation) (the method of Ohkava et al.) [15] in brain and liver homogenate. Oxydative modification of proteins was measured by determination of carbonil group contents in brain and liver homogenate. Blood samples were collected from abdominal aorta. Malondialdehyde (MDA) Protein carbonyls and protein nitrotyrosine were measured in 10% homogenates of liver tissue according to the method of Oliver et al [16].

The body weight and quantity of used food were also registered at the beginning and at the end of experiment. The average body weight of experimental animal was 20.2±2.1g at the start of experiment. There was not statistical difference in body weight between sex (21.3±2.3 females and 19.7±1.9 males) and between experimental and control group. The animals in experimental group exposed to microwave radiation showed less weight gain compare to control (23.2±2.7g vs. 27.1±2.5g; $p<0.05$), after two months. Meanwhile the amount of used food was similar in both groups.

The most important observations were changing of basic behavior models and expression of aggressive or panic behavior. The mice in control group as well as all animals caged in little space showed clear polarization of their living space onto clutch and feeding places. By the rule, these two departments are at the opposite sides of floor diagonal. Instead of this, the experimental animals exposed to microwave field didn't show this spatial organization. Namely, the localization of the clutch was always under an antenna of the mobile phone, even till to feeding places. Collective defense behavior was different in experimental group compared to control. These mice expressed visible individual panic reaction, disorientation and greater degree of anxiety. In control group these deviations of behavior were not register and all animals show compact collective defense reaction.

The histopathological finding in exposed mice was a slightly increased number of micronuclei and discrete perivenular fatty changes in liver.

The exposed mice developed oxidative stress in hepatocites as evidenced by a significant increase in malondialdehyde level (MDA). The lipid peroxide level (MDA) significantly increased about 68% in exposed animals (2.24±0.28 vs control 1.53±0.21 nmol/mg prot.) which may be harmful by accelerating the loss of plasma membrane integrity. Carbonyl group level was significantly increased also (8.23±1.31 vs 6.80 ± 1.34nmol/mg prot. $p<0.05$), capable of altering many cellular functions. The content of MDA in brain tissue is significantly higher (1.42 times) in rats exposed to electromagnetic fields (3,82±0.65 vs. control 2.69±0.42 nmol/mg proteins, $p<0.01$).

IV. Discussion

The widespread use of mobile phones in recent years inevitably raises the questions of the effect of the EM field emitted by such telephones on brain function. A number of reports have now appeared indicating that the high-frequency electromagnetic fields emitted by mobile phones do influence cognitive function and brain electrical activity. It is demonstrated that 30min. human exposure to EM field during sleep reduced waking after sleep onset and affected the EEG in non rapid eye movement (non-REM sleep phase) [10]. This is one of the possible reasons for increased anxiety and slower weight gain of experimental mice [17].

The changing of behavior in experimental animals in the way of losing collective defense reaction and appearance of individual panic reaction couldn't be explained only by disturbance of cognitive functions but also as a result of deep subcortical structures disorders. It is interesting to note that unilateral EMF exposure of the human's head doesn't induce lateralisation of EEG disorders, but symmetrical EEG disorders with both hemispheres involved. Since the thalamus is centrally involved in the generation of sleep spindles, it represents a prime candidate for an EMF sensitive subcortical structure. It is the most likely that fine functional changes in hypothalamus have limited effect in EEG disturbances, which is normalized within 3 hours after exposition to EMF. But the effect of EMF on thalamus seems much stronger attack thalamic functions in domain of reflex and instinctive behavior. On that way disorders of instinctive behavior in experimental animals were more stable and prolonged during a whole day.

Long-term mivrowave radiation increases apoptosis process and induces functional disorders [18,19]. Apoptotic process is best expressed by slightly increased number of micronuclei and discrete perivenular fatty changes [20] which is seen in our study. Cell injury could be result of MR induced oxydative stresslipid peroxidation of unsaturated fatty acids. It is unfavorable process that is followed by disturbances of lipid membranes and other cellular lipid structures. This process leads to massive generation of free radicals and induced membranes protein autooxydation. Results in our study show significant increasing of lipid peroxydation as a direct indicator of hepatocite and brain cell injury. Electromagnetic radiation causes the activation of cellular stress responses, presumably reflecting increased levels of protein structural alteration. In hepatocites and neurons this cause changes in the conformation of biologically active macromolecules and induce disturbance in transport of biomolecules, decreasing detoxycation and cell adaptive mechanisms. Structural alteration of hepatocites and brain cells in experimental animals also include increased contents of carbonyl group, which present sign of altered proteine structure and oxydative stress. Both acute and chronic exposures to microwave radiation altered the function of the cell membrane with increasing permeability and ion flux. Significant increase in ALT (alanine amino trasferase) activity indicates citotoxic effect of non-ionizing radiation on hepatocites inducing apoptosis and necrosis [9]. This is another possible explanation for mechanism of rats' hepatocites injury and DNA damage by EMF.

V. Conclusion

In the experimental group of mice, following effects of exposition to microwave radiation from mobile telephones have been found: increased level of lipid peroxidation and protein oxydative modification. This increased oxidative stress lead to significant disorders of function and structure of brain and liver cell in mice.

It should be noted that experiments with small animals such as mice and rats, must be carefully evaluated for their potential effects when human beings are considered. Despite this fact, reported observations on animals are useful for understanding the corresponding mechanisms in humans.

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