

BIOCHEMICAL AND HISTOPATHOLOGICAL EFFECTS OF MOBILE PHONE EXPOSURE ON RAT HEPATOCYTES AND BRAIN

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Microwave radiation MWR is widespread in human environment. The most frequent sources of MWR are mobile phones and cell towers. The effects of MWR are still unknown and there are insufficient data about long-term MWR effects on hepatocytes and brain structures.

The aim of this paper was to investigate the biological effects of mobile phone microwave radiation on the brain and liver of experimental animals and to determine the increase in oxidative stress as a possible pathogenetic mechanism for harmful effects of long-term exposure.

Wistar rats, three months old, were divided into two groups: I-rats constantly exposed to MWR (3 female and 2 male) and II-control animals without near source of electromagnetic field (EMF) (3 female and 2 male). The microwave radiation was produced by a mobile test phone (model NOKIA 3110; Nokia Mobile Phones Ltd.) connected to a Communication Test Set (model 4202S; Wavetek, Germany). A 900 MHz electromagnetic near-field signal for GSM (Global System for Mobile communication at 900 MHz, continuous wave, analog phone) system was used. The whole-body specific energy absorption (SAR) rate was estimated as 0.025-0.05 W/kg ($E=9.8-18.3$ V/m, $B=4.8-8.6$ μ T). Rats were sacrificed after three months of MWR exposure. The liver and brain were fixed in 10% formaldehyde and paraffin sections were stained by HE. The biochemical analyses comprised the determination of serum activity of AST (Aspartate aminotransferase), ALT (Alanine transaminase), GGT (Gamma-glutamyl transpeptidase) and LDH (Lactate dehydrogenase), as well as determination of serum concentration of sodium, potassium and chloride. Lipid peroxidation was determined by measuring the quantity of malondialdehyde (MDA).

Slightly increased number of micronuclei and discrete perivenular fatty changes were only histopathological findings in the liver of exposed rats. The discrete reduction of gray matter and reduced size and number of dendritic spines of Purkinje cells in cerebellum were notified as well. The serum activity of ALT was significantly increased ($p<0.05$), while activities of AST, GGT and LDH did not change in the exposed rats. Potassium serum concentration was significantly higher in the exposed rats, while the concentration of sodium and chloride did not differ. The MDA concentration was significantly higher in the brain and liver tissues of MWR-exposed rats.

The results in this study show significant increase in lipid peroxidation as a direct indicator of the hepatocytes and brain cells' injury under a long-term (90 days) mobile phone microwave exposure. The hyperkalemia could be the possible systemic marker of impaired cells membrane fluidity and increased permeability, alongside with increased ALT activity as marker of hepatocellular damage. Disorders of hypothalamo- hypophyseal axis lead to disturbances in affective behaviour, but also to disturbances of neurovegetative functions, which leads to behavioral changes and increased appetite and weight gain in exposed animals. *Acta Medica Mediana* 2010;49(1):37-42.

Ključne reči: mobile phones, microwave radiation, GSM, oxidative stress, liver, brain

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Introduction

Non-ionizing radiation (NIR) is widespread in human environment. The most frequent sources of NIR are mobile phones and cell towers which emit microwave radiation (MWR). They emit powerful electromagnetic field (EMF), the effects of which have been unknown yet.

It is generally considered that humans are immune to adverse influences of GSM (Global

System for Mobile Communication) radiation, because it is believed that its intensity is far too low to cause any deleterious degree of body tissue heating, as quantified through the so-called specific absorption rate, or SAR. It is generally believed that for humans adverse effects can arise only from excessive heating.

The low intensity, pulsed microwave radiation currently used in GSM telephony can exert subtle, non-thermal influences on the human organism. This is the consequence of a variety of human organisms' oscillatory electrical biological activities, each characterized by a particular frequency, some of which happen to be close to those used in GSM!

Much experimental evidence of non-thermal influences of MWR on living systems has been published in the peer reviewed scientific literature

during the last 30 years. 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 oxidative stress (1). Also, increased permeability of the erythrocyte membrane and increased hemolisation (2), effects on blood brain-barrier, increase in calcium ion efflux (3), reduced efficiency of lymphocyte cytotoxicity and increase of chromosome aberrations and micronuclei in human blood lymphocytes (4), synergistic effects with cancer promoting drugs such as phorbol ester (5) and impairment of reproductive system (6) were registered.

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 MR, increase in embryo mortality in rats, increased permeability of the blood-brain and hepatocytes membranes in rats, increases in DNA single and double strand breaks in rats, micronuclei formation in the liver and brain tissue in rats (7), neurotransmitter balance, cognitive function, and sleep have recently been reviewed (8). Early effects are minimal and they could be expressed as genetic damage, disturbances of enzymes activity, histological changes etc (9). However, there are still insufficient data about long-term MWR effect on intensity of oxidative stress and damage of hepatocytes and brain tissue.

Aim

The aim of the paper was to investigate the biological effects of mobile phones microwave radiation on the brain and liver of experimental animals and determination of increased oxidative stress as a possible pathogenetic mechanism for harmful effects of long-term exposure.

Animals and Methodology

Experiments were performed on adult Wistar Albino rats bred at the Vivarium of the Institute of Biomedical Research, Faculty of Medicine in Niš, under conventional laboratory conditions. Wistar rats, 3 months old, were divided into two groups: I-rats constantly exposed to MWR (3 female and 2 male) and II-control animals without near source of EMF (3 female and 2 male).

All animals in control and experimental group were housed collectively in polycarbonate cages 30x40x40cm (WxLxH) and given *ad libitum* access to standard laboratory food and water. The housing room was maintained at 24°C with 42±5% relative humidity and had a 12-12-h light-dark cycle (light on 06:00-18:00 h).

Experimental group was continually exposed to MWR from mobile phones. The microwave radiation was produced by a mobile test phone (model NOKIA 3110; Nokia Mobile Phones Ltd.) connected to a Communication Test Set (model 4202S; Wavetek, Germany). A 900 MHz electromagnetic near-field signal for GSM (Global System

for Mobile communication at 900 MHz, continuous wave, analog phone) system was used. The whole-body specific energy absorption (SAR) rate was estimated as 0.025-0.05 W/kg ($E=9.8-18.3$ V/m, $B=4.8-8.6$ μ T, $H=3.7-6.9$ mA/m, $P=1.3-2.7$ mW). The measurement of EMF parameters was done by AARONIA AG Germany, Spectranplus HF 6080.

The mobile telephone was situated in the center of the cage, while the distance of MWR generator from the floor was 3cm and maximal distance from the floor corners was 28.2 cm.

Rats were sacrificed after 3 months. Rats were anesthetized with ketamine HCl (50 mg/kg), administered intraperitoneally (*i.p.*), before sacrificing each rat and removing the brain. The liver and brain tissue were fixed in 10% formaldehyde. Paraffin sections were stained by HE. Biological effects were determined by observation of individual and collective behavior and body mass changes. The body weight and quantity of used food were also registered at the beginning and at the end of experiment.

The intensity of lipid peroxidation in the brain and liver tissue was spectrophotometrically measured, based on the thiobarbituric (TBA) response products from Ohkawa et al. (1979). Homogenate absorption was measured at 532 nm. Malondialdehyde (MDA) – lipid peroxidation end product, concentration was expressed per mg/protein, using the molecular extinction coefficient of MDA (1.56×10^{-5} mol cm^{-1}). Brain proteins were determined according to Lowry's method (Lowry et al., 1951), using bovine serum albumin as standard.

The blood from aorta was collected in plastic heparinised tubes for biochemical analyses. The biochemical analyses comprised determination of serum activity of AST (Aspartate aminotransferase), ALT (Alanine transaminase), GGT (Gamma-glutamyl transpeptidase) and LDH (Lactate dehydrogenase), as well as determination of serum concentration of sodium, potassium and chloride.

Statistical analysis

Data were analyzed using a commercially available statistics software package (SPSS® for Windows, v. 9.0, Chicago, USA). Results were presented as means \pm SD. Statistical significance was determined at level of $p < 0.05$ using the Student's t-test.

Results

The animals exposed to MWR from mobile phone exhibited more aggressive behavior and faster body weight gain than control non-exposed animals ($\Delta\%$ 60.9 vs. 52). Switching on the mobile phone induced rising of animals and their intention to be above the antenna level. These rats expressed also greater degree of anxiety. The average weight of animals during the study is shown in Table 1.

The only histopathological finding in MR-exposed rats was a slightly increased number of micronuclei and discrete perivenular fatty changes

Table 1. Weight gain during the study

Body weight (g)		Beginning of study	End of study	$\Delta\%$
Exposed group	females/males	190/200	306/321.5	61/60.7
	total	194 \pm 12	312.2 \pm 48.5	60.9
Control group	females/males	192/203	292/308	52/51.7
	total	196.4 \pm 14	298.4 \pm 35.2	52

Table 3. The effects of mobile phone MWR on serum concentration of liver enzymes

U/L	ALT	AST	GGT	LDH
Exposed group	66.5 \pm 10.4	175.5 \pm 17.1*	5.75 \pm 0.95	312.75 \pm 86.3
Control group	63.25 \pm 10.5	154.2 \pm 38.1	5.75 \pm 0.5	278.5 \pm 56.5

Data are presented as mean \pm SD; *p<0.05 vs. control

AST (Aspartate aminotransferase), ALT (Alanine transaminase), GGT (Gamma-glutamyl transpeptidase) and LDH (Lactate dehydrogenase),

in the liver. In the brain tissue slices there were not evident pathohistological changes, but discrete reduction of grain mass and reduced size, and number of dendritic spines of Purkinje cells in cerebellum were noticed.

The effects of MWR exposure on serum electrolytes are shown in Table 2.

The MWR induces a significant increase in serum potassium concentration (p<0.05) compared to controls. The concentrations of sodium and chloride did not differ significantly between exposed and control groups (Table 2).

The effects of MWR exposure on liver tissue and serum concentration of liver enzymes are shown in Table 3.

Significant changes in ALT, GGT and LDH serum activity were not registered during a long-term exposure (3 months) to MW radiation. The activity of AST significantly increased in the serum of rats exposed to MW radiation (p<0.05) (Table 3).

The intensity of lipid peroxidation measured by levels of MDA in the liver tissue is shown on Graph 1.

The rats exposed to electromagnetic field develop oxidative stress in hepatocytes as evidenced by a significant increase in malondialdehyde level (MDA). The lipid peroxide level (MDA) significantly increased by 1.46 in exposed animals (2.24 \pm 0.28 vs control 1.53 \pm 0.21 μ mol/g prot.) which may be harmful by accelerating the loss of hepatocyte plasma membrane integrity (Graph 1).

Table 2. The effects of MWR exposure on serum electrolytes concentration

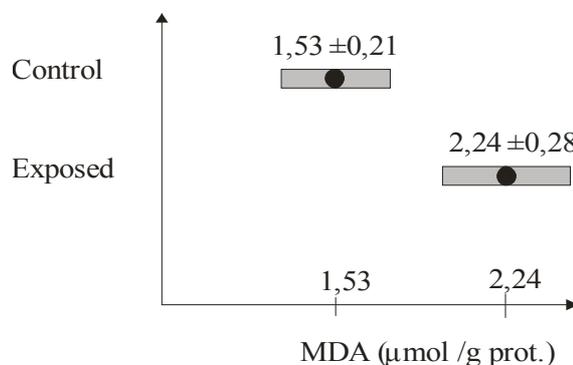
	Na	K	Cl
Exposed group	151.25 \pm 0.9	4.15 \pm 0.25*	109 \pm 3.36
Control group	153.5 \pm 4.65	3.77 \pm 0.26	109.5 \pm 2.38

Data are presented as mean \pm SD; *p<0.05 vs. control

The intensity of lipid peroxidation measured by levels of MDA in the brain tissue is shown on Graph 2.

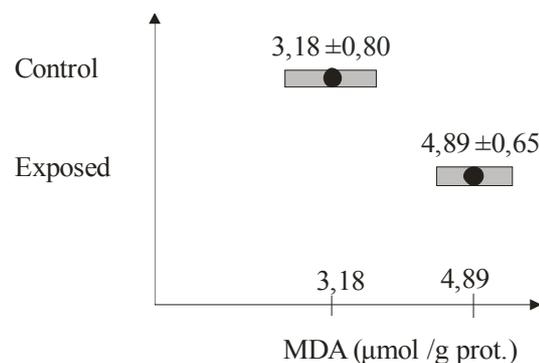
The content of MDA in the brain tissue was significantly higher (1.53 times) in the rats exposed

to electromagnetic field (4,89 \pm 0.65 vs. control 3.18 \pm 0.8 μ mol/g proteins, p<0.01) (Graph 2).



Data are presented as mean \pm SD; *p<0.05 vs. control

Graph 1. The intensity of lipid peroxidation in liver tissue



Graph 2. The intensity of lipid peroxidation in the brain tissue

Discussion

The animals exposed to MWR from mobile phone exert more aggressive behavior and faster body weight gain than control non-exposed animals (Table 1). Some brain structures (globus pallidus, substantia nigra, hypothalamus) are especially amenable to increased production of hydroxyl radicals (OH \cdot). Meanwhile, brain tissue are deficient of antioxidative enzymes such as superoxide dismutase-SOD, glutathion peroxidase-GSH-Px i catalase-CAT. The distribution of these

enzymes is unequal in different brain parts and it is age-dependent (10, 11).

This could indicate possible pathogenetic mechanisms of increased sensitivity to MWR of some central nervous system (CNS) regions, which is primarily expressed as disorders of hypothalamo-hypophyseal functions (12). Disorders of hypothalamo-hypophyseal axis lead to disturbances in affective behaviour, but also to the disturbance of neurovegetative functions. This could explain the reported behavioural changes and increased appetite and weight gain in exposed animals. In particular, a variety of neurological effects have been postulated to EMF, including headaches attributable to the use of mobile phones and changes in sleep patterns (13).

The histopathological findings of discrete reduction in grain mass and slightly reduced size and number of dendritic spines of Purkinje cells in the cerebellum are in line with literature data (14). Neuronal membranes are rich in polyunsaturated fatty acids which are responsible for neuronal integrity and function. This is the main reason for their increased vulnerability to free radicals generation. It is shown that the first changes occurred at the neuronal structure, followed later by glial and endothelial cell damages. It has been shown that increased MDA content had good correlation with the degree of brain tissue damage (15).

Long-term MWR increases cell apoptosis and induces functional disorders in many cell types, which even could be the future approach in cancer treatment (16,17). Apoptotic process is best expressed by slightly increased number of micronuclei and discrete perivascular fatty changes, which is also seen in our study.

Increased potassium serum concentration in MWR-exposed rats (Table 2) could be an indicator of cellular membrane damage. Probably, this is the consequence of increased membrane permeability and potassium leaking, induced by oxidative damage or by impaired function of ion channels (18).

Serum activities of ALT, GGT and LDH did not change significantly after the long-term exposure to MWR. However, there was a significant increase of AST activity in the serum of exposed rats (Table 3). It has become evident that NIR similar to those from mobile phones are capable of exerting a direct effect at cellular and subcellular levels by destabilizing cell membranes and affecting signal transduction pathways. The direct effect of this process is leaking of cytosolic enzymes and energy metabolism disturbances in hepatocytes. 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 activity indicates a cytotoxic effect

of non-ionizing radiation on hepatocytes inducing apoptosis and necrosis as previously described. An elevated oxyradical generation and, subsequently, cell membrane disruptions were the reasons for electromagnetic fields inducing cell damages (19).

This study provides important findings related to oxidative stress in the brain and liver of animals exposed to mobile phones. We demonstrated that mobile phones caused oxidative damage in the brain and liver biochemically by increasing the levels of lipid peroxidation and MDA concentration (Graphs 1 and 2).

The nervous system is particularly vulnerable to ROS due to its high metabolic rate, its deficient oxidant defense mechanisms and its diminished cellular turnover. Sokolović et al. (2009) reported the evidence for neuronal damage caused by non-thermal microwave exposure and increased lipid peroxidation and oxidative protein modification. (20). The lipid peroxidation in these conditions is of particular interest. There are both direct and indirect effects of lipid peroxidation. The direct effects which are the consequence of lipid peroxidation in cell membranes include the loss of fluidity, decrease in electrical resistance, depression in protein mobility in the membrane and increased phospholipid exchange between the bilayers of the membrane (21). Indirect effects of lipid peroxidation are possibly less apparent but likely no less detrimental. The aldehydes that are produced as a consequence of lipid peroxidation are biologically active. One of these aldehydes, i.e. malonaldehyde (MDA) has been shown to cross-link and aggregate membrane proteins. In general, the complex reactions of free radicals, aldehydes, and other products of lipid peroxidation lead to the major destruction of membrane proteins. MDA is the breakdown product of the major chain reactions leading to the oxidation of polyunsaturated fatty acids, and thus serves as a reliable marker of oxidative stress-mediated lipid peroxidation in the rat brain (22).

Conclusion

The results in our study show a significant increase in lipid peroxidation as a direct indicator of hepatocytes and brain cell injury under a long-term (90 days) mobile phone microwave exposure. The hyperkalemia could be a possible systemic marker of impaired cell membrane fluidity and increased permeability, alongside with increased ALT activity as a marker of hepatocellular damage. Disorders of hypothalamo-hypophyseal axis lead to disturbances in affective behaviour, but also in disturbances of neurovegetative functions. This could explain the reported behavioural changes, increased appetite and weight gain in exposed animals.

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BIOHEMIJSKE I PATOHISTOLOŠKE PROMENE MOŽDANOG I JETRINOG TKIVA PACOVA IZAZVANE ZRAČENJEM MOBILNIH TELEFONA

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Mikrotalasno zračenje (MWR) je široko rasprostranjeno u savremenom ljudskom okruženju, a najčešći izvori su mobilni telefoni i repetitori mobilne telefonije. Efekti MWR na žive organizme još uvek su nepoznati i za sada još uvek ne postoji dovoljno podataka o dugotrajnim efektima na hepatocite i moždane strukture.

Cilj rada bio je da se ispituju biološki efekti mikrotalasnog zračenja mobilnih telefona na mozak i jetru eksperimentalnih životinja i odredi intenzitet oksidativnog stresa kao mogućeg patogenetskog faktora za nastanak štetnih efekata u uslovima dugotrajne ekspozicije.

Wistar pacovi, stari 3 meseca, bili su podeljeni u dve grupe: I-pacovi konstantno izloženi MWR-u (3 ženke i 2 mužjaka) i II-kontrolna grupa bez bliskih izvora MWR (3 ženke i 2 mužjaka). Kao izvor mikrotalasnog zračenja poslužio je mobilni test telefon (model NOKIA 3110; Nokia Mobile Phones Ltd.) povezan sa komunikacionim test setom (model 4202S; Wavetek, Germany). Signal za GSM (Globalni Sistem Mobilne komunikacije) frekvencije 900 MHz, kninuranog talasa, analogne telefonije) korišćen je za zračenje. Specifični koeficijent apsorpcije preračunat na celo telo (SAR) iznosio je 0.025-0.05 W/kg ($E=9.8-18.3$ V/m, $B=4.8-8.6$ μ T). Pacovi su žrtvovani nakon tri meseca izlaganja MWR-u. Tkivo jetre i mozga je fiksirano u 10% formaldehidu, a parafinski isecci su bojani hematoksilin-eozinom. Biohemijska analiza je obuhvatila određivanje serumske aktivnosti alanin transferaze (ALT), aspartat transaminaze (AST), gama glutamil transferaze (GGT) i laktat dehidroge-naze (LDH). Elektroliti u serumu su obuhvatili određivanje natrijuma, kalijuma i hlorida. Step en lipidne peroksidacije je određivan merenjem količine malondialdehida (MDA).

Blago povećan broj mikronukleusa i diskretne perivenularne masne promene bile su jedini histopatološki nalaz na jetri eksponiranih pacova. Diskretna redukcija sive mase i blaga redukcija broja i dendritičnih snopova Purkinjeovih ćelija bile su jedini vidljivi nalaz na moždanom tkivu eksponiranih pacova. Serumska aktivnost ALT bila je značajno povećana, dok se vrednosti AST, GGT i LDH nisu menjale u uslovima MWR ekspozicije. Serumska koncentracija kalijuma bila je značajno veća u stanjima ekspozicije dok se vrednosti natrijuma i hlorida nisu značajnije menjale. Vrednosti MDA bile su značajno povećane u jetri i mozgu pacova izloženih MWR-u.

Ekspozicija mikrotalasnom zračenju mobilnih telefona u trajanju od 3 meseca dovela je do značajnog porasta lipidne peroksidacije kao direktnog pokazatelja oštećenja hepatocita i moždanog tkiva. Hiperkaliemija može predstavljati sistemski marker poremećaja permeabilnosti ćelijskih membrana paralelno sa porastom ALT aktivnosti usled hepatocelularnog oštećenja. Poremećaji hipotalamo hipofizne osovine su najverovatniji uzrok neurovegetativnih i poremećaja ponašanja; čime se može objasniti porast apetita, razdražljivost i brži prirast mase ekponiranih životinja. *Acta Medica Medianae* 2010;49(1):37-42.

Ključne reči: mobilni telefon, mikrotalasno zračenje, GSM, oksidativni stres, jetra, mozak