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Review

BIOLOGICAL INDICATORS IN RESPONSE TO RADIOFREQUENCY/MICROWAVE EXPOSURE

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Over the years, due to rapid technological progress, radiation from man-made sources exceeded that of natural origin. There is a general concern regarding a growing number of appliances that use radiofrequency/microwave (RF/MW) radiation with particular emphasis on mobile communication systems. Since non-thermal biological effects and mechanisms of RF/MW radiation are still uncertain, laboratory studies on animal models, tissues, cells, and cell free system are of extraordinary importance in bioelectromagnetic research. We believe that such investigations play a supporting role in public risk assessment. Cellular systems with the potential for a clear response to RF/MW exposures should be used in those studies. It is known that organism is a complex electrochemical system where processes of oxidation and reduction regularly occur. One of the plausible mechanisms is connected with generation of reactive oxygen species (ROS). Depending on concentration, ROS can have both beneficial and deleterious effects. Positive effects are connected with cell signalling, defence against infectious agents, and proliferative cell ability. On the other hand, excessive production, which overloads antioxidant defence mechanism, leads to cellular damage with serious potential for disease development. ROS concentration increase within the cell caused by RF/MW radiation seems to be a biologically relevant hypothesis to give clear insight into the RF/MW action at non-thermal level of radiation. In order to better understand the exact mechanism of action and its consequences, further research is needed in the field. We would like to present current knowledge on possible biological mechanisms of RF/MW actions.

KEY WORDS: *interactions, macromolecules, non-ionizing, non-thermal, radiation, reactive oxygen species*

For centuries human population has been exposed to natural electromagnetic sources such as radiation from sun, space, and earth. Today, besides this natural radiation and owing to rapid technological progress we are more than ever exposed to man-made electromagnetic radiation (EM). Various products and applications in our everyday life make use of some form of electromagnetic energy (1). Connection between technological development and increase in EM radiation doubtlessly exists. Levels of radiation have rapidly increased and man-made sources at specific frequencies now by far exceed those of natural origin. There is a particular concern regarding an increase in the number of appliances which use RF/MW radiation. Some of them have become an integral part of our lives like mobile phones, television, microwave ovens, medical devices, radars, and satellites (2). Besides the obvious benefits, there is also a widespread public concern about the potential health effects this technology can have on biological system and living beings. EM radiation can be described as the propagation of energy through space in the form of waves or particles (photons) and it is characterised by specific wavelength, frequency, and energy. Because of thermal agitation of charged particles every single object is continuously generating electromagnetic field. Energy of the field is directly proportional to its frequency, with higher frequency meaning greater energy (3). Minimum energy capable of causing ionisation by breaking the intermolecular bonds and releasing electrons from an atom or molecule is considered to be 10 eV (1). Based on their ability to cause ionisation, we can distinguish two types of electromagnetic radiation; ionising and non-ionising radiation. Non-ionising radiation includes three frequency ranges; static (0 Hz) and extremely low frequency range (<300 Hz), intermediate frequency range (300 Hz-10 MHz), and radiofrequency range including RF and microwaves (10 MHz to 300 GHz) (2). Over the years, rapid increase in technology, especially telecommunication, has raised great concerns regarding possible adverse health effects of excessive RF/MW exposure. This is not surprising if we know that charged ions, molecules, and structures inside the body contribute to electromagnetic processes that are crucial for the normal functioning of every living organism. These processes are characterised by specific frequencies usually found in the microwave region of electromagnetic field (4).

Passing through a biological system, RF radiation can be reflected, transmitted, refracted or absorbed (1). Absorption of RF energy stirs up the motion of charged particles and rotation of molecules, primarily water, inevitably rising the temperature (5). Measure of absorbed energy within the organism is defined as the specific absorption rate (SAR) and is usually expressed in watts per kilogram (W kg^{-1}) (6). For general public, SAR limit for the whole body is 0.08 W kg^{-1} and for people occupationally exposed to RF energy limit is 0.4 W kg^{-1} (7). Based on the amount of absorbed energy within the biosystem, effects can be divided into thermal and non-thermal. Certainly the most acceptable mechanism of RF interaction is tissue heating. Thermal effects occur with the temperature increase exceeding 1 °C causing cellular and intracellular changes particularly at molecular level (1). The amount of heat depends on the radiation intensity, electrical properties of exposed tissue and body′s thermoregulatory mechanism (7). The World Health Organization stated that many biological effects of acute exposure to non-ionising radiation are consistent with responses to induced heating, resulting either in the rises in tissue or body temperature by about 1 °C or more, or in responses to minimising the total heat load. However, during low intensity radiation thermal homeostasis remains stable due to activated thermoregulation processes. The body temperature might rise by up to 1 °C without accumulation. Based on this statement basic exposure limits for occupational and general population have been established and are known as exposure standards (8). Consequently, the problem of a prolonged exposure to low intensity radiation and non-thermal biological effects derived from RF/MW sources remains open to science.

Biological mechanisms of RF/MW action

A number of published papers point at different modifications and biological effects caused by RF/MW radiation, while at the same time others lack significance. These contradictions are not surprising given the variety of factors influencing experimental procedure. Some of them include polarisation, duration of exposure, continuous or pulsed wave exposure, biological factors of exposed system and many others. All these factors have to be considered so that research results can be reproducible as it was proposed by Belyaev et al. (9) in 2000 and 2005 (10).

The exact mechanism of non-thermal electric and magnetic field interactions with biological structures is still unknown and many different theories suggest various explanations. Detectible changes can only be observed if the applied RF/MW radiation exceeds thermal noise caused by constant random movement of charges at temperatures above absolute zero. Minimum energy required for these processes should exceed 26 meV, the average energy of thermal noise measured at body temperature. Considering low energy level of RF/MW radiation, it is believed that changes could only be detected when biological system is resonantly sensitive to applied frequency (11).

Fröhlich was the first to propose a model based on vibration interactions between large molecules and components of biological systems. Trying to explain possible biological effects he suggested a particular coherent state of vibrations and the existence of specific frequencies within the RF/MW band of electromagnetic spectrum in which energy could be absorbed (12, 13). Under special conditions during vibration, electrically polar structures generate EM field. However significant effects can only be detected when these structures are in resonance with external electric field having the same frequency (11). Because of its ability to detect and amplify small signals against the background noise, organism could be easily compared with a radio receiver. Based on this mechanism, Hyland (4) also suggested the possibility of biological effects occurring at frequencies of mobile phones. These effects could only be observed if certain biological structures had the same vibration frequencies as the applied EM field (12, 14). Cell membranes were first considered a possible source of cellular vibrations until the discovery of cytoskeleton when much attention was given to microtubules. Microtubule reassembly was related with the peak of cellular EM field emission during replication suggesting the crucial role in their generation. Interestingly microtubules have the ability to vibrate in kHz to GHz frequency region (11).

Pokorný et al. (15, 16) proposed a model based on microtubule vibrations. However there were few remarks regarding potential vibration damping given the highly viscous medium surrounding them. Taking this into account, Adair (17) calculated and concluded that because of damping effect vibrations were too small to cause any biological effect. On the other hand, Pokorný (18) showed that damping effect on microtubules could be minimised due to a slip layer formed at the boundary of microtubules and cytosol. Changes in microtubule structure were also observed in several papers (19-22).

Bohr and Bohr (23, 24) suggested that some protein conformations had very similar energies and that transition between them corresponded to the frequencies of approximately few GHz. Based on the experimental results they concluded that MW radiation has the ability to change protein conformation by enhancing the kinetics of protein folding and denaturation, which could eventually lead to detrimental biological effects. De Pomerai et al. (25) also showed that aggregation of protein bovine serum albumin could be enhanced by microwave radiation. Other different approaches have been considered regarding the interactions between RF/MW radiation and biological systems. One of them is based on the interactions with small particles of ferromagnetic material magnetite. Magnetite is found not only in certain bacteria but also in human beings, especially in the brain. It strongly absorbs radiation at frequencies between 500 MHz and 10 GHz and converts the energy into acoustic vibrations dissipating it in cellular structures at microwave frequencies. These findings also led Kirschvink (26) to propose magnetite as a possible mechanism of interaction. Besides magnetic nanoparticles, there are other possible targets for magnetic field interactions like spinning magnetic moments in radical pairs or long-lived rotational states of some molecules (27, 28).

Radical pair mechanism is considered the most probable mechanism in explaining the interactions between cellular and static or extremely low EM field (ELF). At the same time researchers suggest that the proposed mechanism could also explain biological effects of radiofrequency radiation (1, 29). Radical pairs are formed during normal metabolic processes and although have very short lifetime they react with each other or other molecules and generate new radicals. They contain one or more unpaired electrons and their magnetic properties are related to electron spin. Electron spins of two radicals can be in either singlet or triplet state i.e. parallel or opposite from one another. Oscillations between two states depend on magnetic interactions of unpaired electrons with each other, nuclear spins, and external magnetic field (30). Stable molecules are formed only when two spins are opposite one from the other, while parallel spins first require a spin change. External magnetic field slows down the processes of spin change, causes radical pair separation and increases their lifetime and concentration. The process is even more expressed when radicals are attached to cellular structures such as cell membrane (31). A biophysical model for the effect of oscillating electric fields on cells has been given by Panagopoulos et al. in 2002. This model is based on forced-vibration of free ions on the surface of a plasma membrane caused by an external oscillating field. It was shown that coherent vibration of electric charge was able to affect electro sensitive channels on the membrane which could lead to the disruption of cell′s electrochemical balance and function. The proposed model theoretically proved that pulsed EM fields could be twice as effective biologically as the continuous ones (32). Furthermore there have been a number of papers connecting exposure of RF/MW radiation with excessive formation of free radicals (67-74). Action of free radicals is additionally enhanced in the presence of oxygen which further causes the formation of new radicals. These free radicals are also known as reactive oxygen species (33).

Reactive oxygen species

Under physiological conditions, ROS are generated everyday as a result of various metabolic processes. They are known to have both beneficial and deleterious effects. At low concentrations they are often involved in defence against infectious agents, cell signalling, and mitogenic response. On the other hand, overload of ROS concentration and antioxidant deficiency leads to cellular damage including membranes, lipids, proteins, and even DNA. Several diseases are associated with adverse effects caused by reactive oxygen species such as diabetes, atherosclerosis, chronic inflammation, malignant and neurodegenerative diseases, and many others (34, 35).

Balance between these effects is accomplished by redox regulation (34, 36). ROS include oxygen radicals like superoxide radical ($O_2^{\cdot-}$), hydroxyl ($HO^{\cdot}$), peroxyl ($RO_2^{\cdot}$), and alkoxyl ($RO^{\cdot}$) radicals as well as nonradical derivatives of oxygen like hydrogen peroxide (H_2O_2), hypochlorous acid (HClO), and ozone (O_3) (37).

Even oxygen molecule, having two unpaired electrons found in triplet state, qualifies as a free radical, or to be precise - biradical. However, because of parallel spins oxygen is able to accept only one electron at a time which consequently slows down its′ reactivity (Figure 1) (38).

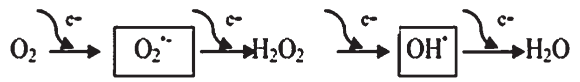

Figure 1 *One electron reduction of oxygen and formation of superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) with water as a final electron acceptor.*

One of the major sources of ROS in the cell is mitochondrion (35). Out of total oxygen consumed, 1 % to 2 % is converted to oxygen free radicals, mostly at the level of complex I and complex III of respiratory chain (39, 40). Besides mitochondrion, there are several other sources of ROS in the cell. Some of them include peroxisomes, enzymes like xanthine oxidase, cytochrome P450, neutrophils, eosinophils, and macrophages (41, 42). Superoxide radicals are mostly generated in mitochondria as a result of electron leakage from electron transport chain and premature reduction of oxygen. They are known as the primary ROS able to generate "secondary" ROS by further reacting with other molecules. Superoxide radicals extract iron from iron-containing molecules and enable Fe^{2+} to participate in the Fenton reaction creating hydroxyl radical (Eq. 2). They also participate in the Haber-Weiss reaction (Eq. 3), which combines the reduction of iron and the Fenton reaction (Eq. 1 and 2) (43).

$$Fe^{+3} + O_2^{\cdot-} = O_2 + Fe^{+2} \quad (1)$$

$$Fe^{+2} + H_2O_2 = Fe^{+3} + HO^{\cdot} + OH^{-} \quad (2)$$

Equation 1, 2 - the Fenton reaction

$$O_2^{\cdot-} + H_2O_2 = O_2 + HO^{\cdot} + OH \quad (3)$$

Equation 3 – the Haber-Weiss reaction

Hydroxyl radicals can also be produced in reaction with other metals like copper, chromium or cobalt (44). However, reaction catalysed by iron is considered to be the major mechanism in the generation of highly reactive hydroxyl radicals (45). Because of a very short lifetime, these radicals are known to react in close proximity of the site of formation (43). Superoxide can also generate hydrogen peroxide in a reaction of dismutation. In biological systems, this reaction is additionally accelerated by superoxide dismutase (SOD) (46). Although not a radical, hydrogen peroxide is considered to be a reactive oxygen metabolite. It can easily pass the cell membrane and directly or indirectly cause damage to macromolecules even at a low concentration. Because of its lipid solubility H_2O_2 can also generate $HO^{\cdot}$ radical at localised sites containing iron or copper (47).

Excessive level of free radicals can cause severe damage to biological systems, which could eventually lead to disease development. Because of their high level of polyunsaturated fatty acids (PUFAs), cell membranes are particularly sensitive to oxidative damage. During the three-stage process of lipid peroxidation, aldehydes are formed as a final product (48). One of the most abundant products of lipid peroxidation is certainly malondialdehyde (MDA), which is also used as a biomarker of oxidative stress. MDA is a known mutagen and carcinogen. It reacts with DNA and forms adducts with deoxyguanosine, deoxyadenosine, and deoxycytidine (49, 50). Besides lipids, proteins are also highly sensitive to free radical exposure. They are involved in different biological functions and even the slightest change in protein structure or conformation could result with their loss of function. Modifications of amino acids, fragmentation of polypeptide chains or protein-protein cross-linkage are some of the changes that could be induced as a result of protein interactions with free radicals (51). Considering that different modifications are numerous, there are also various methods for detecting and quantifying them. Certainly the most measurable products of protein oxidation are protein carbonyls (52). They are characterised by carbonyl group generated at the protein side chains. Because of their stability, they can be easily detected (53). ROS can also react with nucleic acids, especially DNA, and cause different modifications, even single or double strand breaks. Guanine is most susceptible to oxidative damage and in reaction with ROS forms highly mutagenic and carcinogenic 8-oxoguanine (43). Because of a high level of ROS present in mitochondria and limited repair mechanism, mitochondrial DNA is particularly sensitive to free radicals (54).

Cell antioxidant defence system

Given the variety of possible damage that could be caused by free radicals, organisms have developed a whole spectrum of different defence mechanisms. They include preventive and repair mechanisms as well as physical and antioxidant defence. Antioxidant defence includes enzymes like SOD, glutathione peroxidase (GPx), and catalase (CAT), while non-enzymatic antioxidants include glutathione (GSH), vitamin E and C, carotenoids, flavonoids, and many others (34). Certainly one of the most significant compounds of antioxidant defence is glutathione. Glutathione is a low molecular weight antioxidant capable of preventing oxidative damage by reacting directly or indirectly with ROS (47). It is found in reduced (GSH) or oxidised (GSSG) form with their ratio often used as a measure of toxicity or antioxidant capacity (55). GSH can react directly with free radicals. This results in GSH becoming a radical itself that forms GSSG reacting with another GSH radical. Accumulation of GSSG causes disturbance of redox balance, which affects metabolic processes, homeostasis, and cellular integrity (56, 57). GSH is also known to act as a cofactor for several enzymes, participates in the transport of amino acids and helps the regeneration of vitamin E and ascorbic acid (47, 43).

As previously mentioned, SOD helps to remove superoxide radicals by producing hydrogen peroxide which is later removed by the activity of GPx and CAT. There are three SOD isoforms that exist in animal tissues; cytosolic CuZnSOD, MnSOD, and extracellular SOD (ECSOD), each dealing with decomposition of $O_2^{\cdot -}$ in different compartments (58).

DISCUSSION

It is known that altering magnetic field produces an electric field and vice versa, a varying electric field generates a magnetic field. Because of this interdependence, both fields jointly are considered a single entity - the electromagnetic field (59). Alternating electric fields have a wide range of effects on living systems. At extremely low frequencies, electric fields stimulate excitable tissues through membrane depolarisation, stimulate bone growth, and accelerate fracture healing (60-63). As the electric field frequency increases, the stimulatory effect disappears, ending in the so-called thermal effect, when tissue heating becomes a dominant event (64). In-between the desired stimulatory and undesired heating effects, there is this mostly unexplored biological potential of RF/MW radiation at a non-thermal level of exposure.

Since the expanded mobile phone use has caused a serious apprehension worldwide, along with the fact that relationship between low intensity RF/MW radiation and its undesirable bioeffects are very close, we attempted to recognise reasonable indicators which might give an insight into the mechanism of action. It seems that such approach has become more important than ever.

Considering the Fröhlich hypothesis, Pokorný at al. (65) investigated the non-thermal cellular effects of radiation in the microwave range. It was concluded that the observed intracellular changes are a consequence of macromolecular chain resonance caused by external radiation. In electrobiological entity such as a single cell, internal electromagnetic field created by own polar intracellular structures enters into a coherent state with external field, which means that oscillating electric field of low strength could cause significant non-thermal biological changes. As it was mentioned previously, it occurs when the wavelength of radiation is in the range of the properties of biological tissue. In the state of coherence, external field could cause attenuation or amplification of internal frequency field leading to disruption of homeostatic balance. Further, RF/MW radiation seems to induce electric oscillations that disturb cell membrane proteins, activating enzyme cascades that may transfer cell surface signals to the intracellular system (66).

Several investigations point at a possible connection between RF/MW exposure and ROS generation. Results of conducted researches are often contradictory and inconclusive because many factors have to be considered during the experiment. One of the reasonable pathways of RF/MW intracellular action is based on the generation of reactive oxygen species (ROS). Increased levels of ROS, antioxidants, and cellular damage were found in different types of investigations including cell cultures, laboratory animals, and even in the humans after RF/MW exposure (67-74). Xu et al. (75) exposed human lens epithelial cells to 1.8 GHz RF field at different SAR values. After 2 h of intermittent exposure, only the group exposed to smallest SAR value of 1 W kg^{-1} didn't cause increase in ROS concentration, while other groups of 2 W kg^{-1}, 3 W kg^{-1} and 4 W kg^{-1} did. In order to show the effects of cellular phone radiation on the brain and blood of guinea pigs, Meral et al. (76) exposed animals to 890 MHz to 915 MHz radiation for 12 h per day for 30 days. The brain concentration of MDA increased, while CAT and GSH decreased. On the other hand, the blood levels of MDA, CAT, vitamins A, D3, and E increased and GSH decreased. A study conducted by Moustafa et al. (49) examined the effect of acute exposure to 900 MHz radiofrequency field on 12 male volunteers. Results showed significant increase in plasma lipid peroxides and reduction in the SOD and GPx activity in erythrocytes supporting the interaction of radiofrequency fields with biological systems. However, numerous studies were unable to connect generation of ROS and oxidative damage with RF/MW exposure (77-80). In their study, Ferreira et al. (81) exposed rats to 834 MHz radiation to determine the effects on non-enzymatic antioxidant defence, as well as lipid and protein damage. After measuring the level of MDA and protein carbonyls in rat brain tissue, they concluded that there was no significant difference compared to the sham exposed group. Combining the effects of EM radiation and chemicals known to induce free radicals, researchers also tried to investigate the possibility that RF/MW radiation alters the effect of chemical agents. Zmyslony et al. (82) tested the effect of iron ions on rat lymphocytes exposed to continuous wave (CW) EM field. The authors compared two groups of lymphocytes, one treated with $FeCl_2$ and the other treated with $FeCl_2$ and exposed to 930 MHz. They noticed that after acute exposure for 5 min and 15 min ROS level was significantly higher in the exposed group. Similar experiments and results were published by Höytö (83) and Luukkonen et al. (84). It could be concluded that existence of non-thermal effects, including oxidative damage caused by RF/MW radiation, must not be denied. Thereafter, the International Agency for Research on Cancer (IARC) classified RF electromagnetic fields as possibly carcinogenic to human (85). Given the electromagnetic nature of living beings and a growing number of man-made sources of non-ionising radiation, there is also a constant need for better understanding the exact mechanism of interaction between radiation and cellular systems. What makes it particularly difficult is certainly the highly dynamic and complex organisation and function of every organism. A variety of factors contribute to research results, which is the reason why *in vitro* studies are frequently used in this kind of research. However, despite precisely defined and controlled studies, some results are still open to doubt. Future research should pay more attention to determining a possible connection between RF/MW radiation and generation of free radicals *in vitro*. Special consideration should be given not only to the concentration but also to the type of ROS, their origin and the changes in the antioxidant defence system, as well as to macromolecular changes.

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REFERENCES

1. Vecchia P, Matthes R, Ziegelberger G, Lin J, Saunders R, Swerdlow A, editors. Exposure to High Frequency Electromagnetic Fields, Biological Effects and Health Consequences (100 kHz-300 GHz). Oberschleißheim: ICNIRP; 2009.
2. Repacholi MH. An overview of WHO's EMF project and the health effects of EMF exposure. In: Proceedings of the International Conference on Non-Ionizing Radiation at UNITEN (ICNIR 2003). Electromagnetic Fields and Our Health; 20-22 Oct 2003; Kuala-Lumpur, Malaysia [displayed 11 July 2012]. Available at http://www.who.int/peh-emf/meetings/archive/en/keynote4repacholi.pdf
3. Federal Communications Commission Office of Engineering & Technology. Questions and answers about biological effects and potential hazards of radiofrequency electromagnetic fields. OET BULLETIN 56 Fourth Edition, 1999 [displayed 11 July 2012]. Available at http://transition.fcc.gov/Bureaus/Engineering_Technology/Documents/bulletins/oet56/oet56e4.pdf
4. Hyland GJ. Physics and biology of mobile telephony. Lancet 2000;356:1833-6.
5. Challis LJ. Mechanisms for interaction between RF fields and biological tissue. Bioelectromagnetics 2005;Supplement 7:S98-106.
6. Valberg PA. Modulated RF energy: mechanistic viewpoint on the health implications. In: Repacholi M, van Deventer E, Ravazzani P, editors. Proceedings International Workshop on Base Stations and Wireless Networks: Exposures and Health Consequences; 15-16 June 2005; Geneva, Switzerland. Geneva: WHO; 2005. p. 33-46.
7. International Commission on Non-Ionizing Radiation Protection (ICNIRP). Guidelines for limiting exposure to time-varying electric, magnetic, and electromagnetic fields (up to 300 GHz). Health Phys 1998;97:257-8.
8. World Health Organization (WHO). Electromagnetic Fields (300 Hz to 300 GHz). Environmental Health Criteria 137. Geneva: WHO; 1993.

9. Belyaev IY, Shcheglov VS, Alipov ED, Ushakov VD. Nonthermal effects of extremely high-frequency microwaves on chromatin conformation in cells *in vitro* - dependence on physical, physiological, and genetic factors. IEEE Trans Microw Theory Tech 2000;48:2172-9.
10. Belyaev I. Non-thermal biological effects of microwaves. Microwave Rev 2005;11:13-29.
11. Cifra M, Fields JZ, Farhadi A. Electromagnetic cellular interactions. Prog Biophys Mol Biol 2011;105:223-46.
12. Independent Expert Group on Mobile Phones (IEGMP). Mobile Phones and Health, 2000 [displayed 11 July 2012]. Available at http://www.iegmp.org.uk/report/text.htm.
13. Hyland GJ. Physical basis of adverse and therapeutic effects of low intensity microwave radiation. Indian J Exp Biol 2008; 46, 403-19.
14. Pavičić I. Djelovanje radiofrekvencijskog zračenja na strukture citoskeleta stanica u kulturi [Influence of radiofrequency radiation on cytoskeleton structure of cells in culture, in Croatian]. [PhD thesis]. Zagreb: Faculty of Science, University of Zagreb; 2008.
15. Pokorný J, Jelínek F, Trkal V, Lamprecht I, Hölzel R. Vibrations in microtubules. Astrophys Space Sci 1997;23:171-9.
16. Pokorný J, Jelínek F, Trkal V. Electric field around microtubules. Bioelectrochem Bioenerg 1998;45:239-45.
17. Adair RK. Vibrational resonances in biological systems at microwave frequencies. Biophys J 2002;82:1147-52.
18. Pokorný J. Excitation of vibrations in microtubules in living cells. Bioelectrochemistry 2004;63:321-6.
19. Pavičić I, Trošić I. Interaction of GSM modulated RF radiation and macromolecular cytoskeleton structures. In: Kalkan T, Özkan M, editors. 6th International Workshop on Biological Effects of Electromagnetic Fields; 10-14 Oct 2010; Bodrum, Turkey [displayed 11 July 2012]. Available at http://www.istanbul.edu.tr/6internatwshopbioeffemf/cd/pdf/poster/INTERACTION%20OF%20GSM%20MODULATED%20RF%20RADIATION%20AND.pdf
20. Pavičić I, Trošić I. *In vitro* testing of cellular response to ultra high frequency electromagnetic field radiation. Toxicol In Vitro 2008;22:1344-8.
21. Ballardin M, Tusa I, Fontana N, Monorchio A, Pelletti C. Rogovich A, Barale R, Scarpato R. Non-thermal effects of 2.45 GHz microwaves on spindle assembly, mitotic cells and viability of Chinese hamster V-79 cells. Mutat Res 2011;716:1-9.
22. Trošić I, Pavičić I. Disturbance of cell proliferation in response to mobile phone frequency radiation. Arh Hig Rada Toksikol 2009;60:109-15.
23. Bohr H, Bohr J. Microwave-enhanced folding and denaturation of globular proteins. Phys Rev E Stat Phys Plasmas Fluids Relat Interdiscip Topics 2000;61:4310-4.
24. Bohr H, Bohr J. Microwave enhanced kinetics observed in ORD studies of a protein. Bioelectromagnetics 2000;21:68-72.
25. De Pomerai DI, Smith B, Dawe A, North K, Smith T, Archer DB, Duce IR, Jones D, Candido EP. Microwave radiation can alter protein conformation without bulk heating. FEBS Lett 2003;543:93-7.
26. Kirschvink JL. Microwave absorption by magnetite: a possible mechanism for coupling nonthermal levels of radiation to biological systems. Bioelectromagnetics 1996;17:187-94.
27. Binhi VN. Theoretical concepts in magnetobiology. Electromagn Biol Med 2001;20:43-58.
28. Binhi VN, Rubin AB. Magnetobiology: the kt paradox and possible solutions. Electromagn Biol Med 2007;26:45-62.
29. European Commission Joint Research Centre. Reports on theoretical mechanisms and their plausibility, including experimental/epidemiological evidence, computer models and explicit evaluation of contrary arguments [displayed 17 January 2012]. Available at http://ihcp.jrc.ec.europa.eu/our_activities/public-health/exposure_health_impact_met/emf-net/docs/reports/D41_Reports%20on%20theoretical%20mechanisms%20.pdf
30. Stass DV, Woodward JR, Timmel CR, Hore PJ, McLauchlan KA. Radiofrequency magnetic feld effects on chemical reaction yields. Chem Phys Lett 2000;329:15-22.
31. Scaiano JC. Control of chemical reactions with magnetic fields. Interscientia [displayed 11 January 2012]. Available at http://www.uottawa.ca/publications/interscientia/inter.1/magnetic.html
32. Panagopoulos DJ, Karabarbounis A, Margaritis LH. Mechanism for action of electromagnetic fields on cells. Biochem Biophys Res Commun 2002;298:98-102.
33. Panagopoulos DJ. Analyzing the health impacts of modern telecommunications microwaves. In: Berhardt LV, editor. Advances in medicine and biology. New York (NY): Nova Science Publishers; 2011. p. 1-55.
34. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
35. Waris G, Ahsan H. Reactive oxygen species: role in the development of cancer and various chronic conditions. J Carcinog 2006;5:14.
36. Poli G, Leonarduzzi G, Biasi F, Chiarpotto E. Oxidative stress and cell signalling. Curr Med Chem 2004;11:1163-82.
37. Halliwell B, Guttenridge JMC. Free Radicals in Biology and Medicine. 4th ed. New York: Oxford University Press; 2007.
38. Smith C, Marks AD, Lieberman M. Mark's Basic Medical Biochemistry a Clinical Approach. 2th ed. Phyladelphia: Lippincott Williams & Wilkins; 2005.
39. Turrens JF. Mitochondrial formation of reactive oxygen species. J Physiol 2003;552:335-44.
40. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants, and the degenerative diseases of aging. Proc Natl Acad Sci USA 1993;90:7915-22.
41. Inoue M, Sato EF, Nishikawa M, A-M Park, Kira Y, Imada I, Utsumi K. Mitochondrial generation of reactive oxygen species and its role in aerobic life. Curr Med Chem 2003;10:2495-505.
42. Li C, Jackson RM. Reactive species mechanisms of cellular hypoxia-reoxygenation injury. Am J Physiol Cell Physiol 2002;282:C227-41.
43. Valko M, Rhodes CJ, Moncola J, Izakovic M, Mazura M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact 2006;160:1-40.
44. Stohs SJ, Bagchi D. Oxidative mechanisms in the toxicity of metal ions. Free Radic Biol Med 1995;18:321-36.
45. Kehrer JP. The Haber-Weiss reaction and mechanisms of toxicity. Toxicology 2000;149: 43-50.

46. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Mol Cell Biochem 2004;266:37-56.
47. Kohen R, Nyska A. Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. Toxicol Pathol 2002;30:620-50.
48. Del Rio D, Stewart AJ, Pellegrini N. A review of recent studies on malondialdehyde as toxic molecule and biological marker of oxidative stress. Nutr Metab Cardiovasc Dis 2005;15:316-28.
49. Moustafa YM, Moustafa RM, Belacy A, Abou-El-Ela SH, Ali FM. Effects of acute exposure to the radiofrequency fields of cellular phones on plasma lipid peroxide and antioxidase activities in human erythrocytes. J Pharm Biomed Anal 2001;26:605-8.
50. Marnett LJ. Oxy radicals, lipid peroxidation and DNA damage. Toxicology 2002;181-82:219-22.
51. Fagan JM, Sleczka BG, Sohar I. Quantitation of oxidative damage to tissue proteins. Int J Biochem Cell Biol 1999;31:751-7.
52. Shacter E. Quantification and significance of protein oxidation in biological samples. Drug Metab Rev 2000;32:307-26.
53. Dalle-Donne I, Rossi R, Giustarini D, Milzani A, Colombo R. Protein carbonyl group as biomarkers of oxidative stress. Clin Chim Acta 2003;329:23-38.
54. Circu ML, Aw TY. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic Biol Med 2010;48:749-62.
55. Perricone C, De Carolis C, Perricone R. Glutathione-a key player in autoimmunity. Autoimmun Rev 2009;8:697-701.
56. Aw TY. Cellular redox: a modulator of intestinal epithelial cell proliferation. News Physiol Sci 2003;18:201-4.
57. Trošić I, Bušljeta I. Erythropoietic dynamic equilibrium in rats maintained after microwave irradiation. Exp Pathol 2006;57:247-51.
58. Bergamini CM, Gambetti S, Dondi A, Cervellati C. Oxygen, reactive oxygen species and tissue damage. Curr Pharm Des 2004;10:1611-26.
59. International Agency for Research on Cancer (IARC). Non-Ionizing Radiation, Part 1: Static and Extremely Low-Frequency (ELF) Electric and Magnetic Fields. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Vol. 80. Lyon: IARC; 2002.
60. Elwood JM. Epidemiological studies of radio frequency exposures and human cancer. Bioelectromagnetics 2003;Suppl 6:S63-73.
61. Mark SC. Membrane potential perturbations induced in tissue cells by pulsed electric fields. Bioelectromagnetics 1995;16:255-62.
62. McClanahan BJ, Phillips RD. The influence of electric field exposure on bone growth and fracture repair in rats. Bioelectromagnetics 1983;4:11-9.
63. Tsai MT, Chang WH, Chang K, Hou RJ, Wu TW. Pulsed electromagnetic fields affect osteoblast proliferation and differentiation in bone tissue engineering. Bioelectromagnetics 2007;28:519-28.
64. Polk C, Postow E. Handbook of Biological Effects of Electromagnetic Fields. 2nd ed. Boca Raton: CRC Press; 1996.
65. Pokorny J, Jelínek F, Trkal V, Šrobar F. Vibration in microtubules. In: Bersam F, editor. Electricity and magnetism in biology and medicine. Bologna: Kluwer Academic/Plenum Publishers; 1999. p. 967-70.
66. Adey WR. Cell and molecular biology associated with radiation fields of mobile telephones. In: Stone WR, Ueno S, editors. Review of radio science 1996-1999. Oxford: Oxford University Press; 1999. p. 845-72.
67. Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, Musumeci F, Vanella A, Triglia A. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neurosci Lett 2010;473:52-5.
68. Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis 2008;14:964-9.
69. Dasdag S, Bilgin HM, Akdag MZ, Celik H, Aksen F. Effect of long term mobile phone exposure on oxidative-antioxidative processes and nitric oxide in rats. Biotechnol Biotechnol Equip 2008;22:992-7.
70. Ozguner F, Altinbas A, Ozaydin M, Dogan A, Vural H, Kisioglu AN, Cesura G, Yildirim NG. Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. Toxicol Ind Health 2005;21:223-30.
71. Yurekli AI, Ozkan M, Kalkan T, Saybasil H, Tuncel H, Atukeren P, Gumustas K, Seker S. GSM base station electromagnetic radiation and oxidative stress in rats. Electromagn Biol Med 2006;25:177-88.
72. Ilan A, Gurel A, Armutcu F, Kamisli S, Iraz M, Akyol O, Ozen S. Ginkgo biloba prevents mobile phobe-induced oxidative stress in rat brain. Clin Chim Acta 2004;340:153-62.
73. De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and dna damage in human spermatozoa *in vitro*. PLoS One 2009;4:e6446.
74. Marjanović AM. Oksidacijski stres u osoba profesionalno izloženih mikrovalnom zračenju [Oxidative stress in subjects occupationally exposed to microwave radiation, in Croatian] [Graduation thesis]. Zagreb: Faculty of Science, University of Zagreb; 2009.
75. Xu S, Zhou Z, Zhang L, Yu Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Zhong M. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res 2010;1311:189-96.
76. Meral I, Mert H, Mert N, Deger Y, Yoruk I, Yetkin A, Keskin S. Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. Brain Res 2007;1169:120-4.
77. Simkó M, Hartwig C, Lantowa M, Lupke M, Mattsson M-O, Rahman Q, Rollwitz J. Hsp70 expression and free radical release after exposure to non-thermal radio-frequency electromagnetic fields and ultrafine particles in human Mono Mac 6 cells. Toxicol Lett 2006;161:73-82.
78. Khalil AM, Alshamali AM, Gagaa MH. Detection of oxidative stress induced by mobile phone radiation in tissues of mice using 8-oxo-7, 8-dihydro-2'-deoxyguanosine as a biomarker. World Acad Sci Eng Technol 2011;76:657-62.

79. Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. Bioelectromagnetics 2010;31:417-24.
80. Brescia F, Sarti M, Massa R, Calabrese ML, Sannino A, Scarttí MR. Reactive oxygen species formation is not enhanced by exposure to UMTS 1950 MHz radiation and co-exposure to ferrous ions in Jurkat cells. Bioelectromagnetics 2009;7:525-35.
81. Ferreira AR, Bonatto F, de Bittencourt Pasquali MA, Polydoro M, Dal-Pizzol F, Fernández C, de Salles ÁAA, Moreira JCF. Oxidative stress effects on the central nervous system of rats after acute exposure to ultra high frequency electromagnetic fields. Bioelectromagnetics 2006;27:487-93.
82. Zmyślony M, Politanski P, Rajkowska E, Szymczak W, Jajte J. Acute exposure to 930 MHz CW electromagnetic radiation *in vitro* affects reactive oxygen species level in rat lymphocytes treated by iron ions. Bioelectromagnetics 2004;25:324-8.
83. Höytö A, Luukkonen J, Juutilainen J, Naarala J. Proliferation, oxidative stress and cell death in cells exposed to 872 MHz radiofrequency radiation and oxidants. Radiat Res 2008;170:235-43.
84. Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872 MHz radiofrequency radiation. Mutat Res 2009;662:54-8.
85. International Agency for Research on Cancer (IARC). IARC classifies radiofrequency electromagnetic fields as possibly carcinogenic to humans [displayed 11 January 2012]. Available at http://www.iarc.fr/en/media-centre/pr/2011/pdfs/pr208_E.pdf

Sažetak

BIOLOŠKI POKAZATELJI KAO ODGOVOR NA IZLOŽENOST RADIOFREKVENCIJSKOMU/ MIKROVALNOM ZRAČENJU

Zbog ubrzanoga tehnološkog napretka tijekom godina zračenje iz umjetnih izvora mnogostruko je nadmašilo zračenje iz prirodnih izvora. Rastući broj uređaja koji rabe radiofrekvencijsko/mikrovalno (RF/MW) zračenje, osobito mobilni komunikacijski sustav, izaziva zabrinutost opće i znanstvene javnosti. S obzirom na to da su učinci i mehanizmi netermalnoga biološkog djelovanja RF/MW zračenja dvojbeni, laboratorijske studije u kojima se rabe životinjski modeli, tkiva, stanice i bestanični sustavi smatraju se izuzetno važnima u bioelektromagnetskim istraživanjima. Vjerujemo da takva istraživanja podupiru procjenu opasnosti od zračenja za živi organizam. Zbog mogućnosti jasnog odgovora stanice na RF/MW izloženost preporučuje se uporaba staničnog sustava. Zna se da je organizam složen elektrokemijski sustav, gdje se među mnogim drugima redovito događaju oksidacijsko-redukcijski procesi. Jedan od mogućih mehanizama djelovanja povezuje se sa stvaranjem reaktivnih kisikovih spojeva (ROS). Ovisno o koncentraciji ROS-ovi mogu štetiti, ali i djelovati korisno na biosustav. Povoljni učinci ROS-ova povezuju se s diobenom sposobnosti stanice, staničnim signalnim putovima i obranom protiv infektivnih čimbenika. Međutim prekomjerno stvaranje koje preopterećuje antioksidacijski obrambeni sustav dovodi do oštećenja stanica i mogućnosti razvoja bolesti. Čini se da je hipoteza o povišenju koncentracije ROS-ova u stanici zbog zračenja biološki valjana, jer može pojasniti mehanizam netermalnih učinaka RF/MW zračenja. S ciljem boljeg razumijevanja točnog mehanizma, kao i njegovih posljedica potrebna su dodatna istraživanja. Ukratko su prikazane postojeće spoznaje o mogućim biološkim mehanizmima djelovanja RF/MW zračenja.

KLJUČNE RIJEČI: *makromolekule, neionizirajuće zračenje, netermalno zračenje, reaktivni kisikovi spojevi*

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