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# ORIGINAL ARTICLE

# High-frequency electromagnetic radiation and the production of free radicals in four mouse organs

#### Jan BARCAL<sup>1</sup>, Pavel STOPKA<sup>2</sup>, Jana Křížová<sup>2</sup>, Jan Vrba<sup>3</sup>, František Vožeн<sup>1,4</sup>

<sup>1</sup> Charles University Prague, Faculty of Medicine Pilsen, Department of Pathophysiology, Czech Republic; <sup>2</sup> Academy of Sciences of the Czech Republic, Institute of Inorganic Chemistry; <sup>3</sup> Czech Technical University in Prague, Faculty of Electrical Engineering, Department of Electromagnetic Field, Czech Republic; <sup>4</sup> Biomedical Centre, Faculty of Medicine in Pilsen, Charles University in Prague, Plzeň, Czech Republic.

*Correspondence to*: Jan Barcal, Charles University Prague, Faculty of Medicine Pilsen, Department of Pathophysiology, Lidická 1, 301 66 Pilsen, Czech Republic. tel: +420 377593364; fax: +420 377593369; e-mail: jan.barcal@lfp.cuni.cz

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**Abstract**The study was designed to evaluate the effect of high-frequency electromagnetic field (HF<br/>EMF) 900 MHz on reactive oxygen species (free radicals) production. The experimental<br/>animals (10 mice of C3H strain) were exposed to whole-body irradiation over 14 days<br/>(3 hours daily) in a special exposure chamber with the possibility of a specific absorption<br/>rate measurement. The 10 control animals were kept in an analogous position (inside the<br/>chamber) but in turn-off mode. After the last exposure, four tissue samples (brain, liver,<br/>heart, kidney) were immediately stored in liquid nitrogen and transported to a special<br/>electron paramagnetic (spin) resonance spectroscopy measurement. In all four tissue<br/>samples of irradiated animals, a statistically significant increase (p<0.0025) of hydroxyl<br/>radicals concentration was found. These results confirm previous experiments with indi-<br/>rect assessment of free radicals overproduction (made by enzymatic systems depiction)<br/>and strongly support the hypothesis about the possible mechanism and/or harmful effect<br/>of long-term HF EMF exposure.

#### INTRODUCTION

High frequency electromagnetic field (HF EMF) has become a common part of our environment because it is produced by many artificial sources as radars, transmitters and especially cellular (mobile) phones. When considering possible harmful effects, the term "electromagnetic smog" is often used for this situation. Among the sources mentioned, first of all the number of mobile phones is rapidly rising (12 million devices were registered in the Czech Republic in 2011) and during a call the source of radiation is positioned close to the head. That is why there is a question of possible negative effects of HF EMF on a human body, especially on the brain (Repacholi 2001). HF EMF can influence tissues by both thermal and nonthermal effects. While the thermal effect is relatively well known, the non-thermal effects are still under discussion and remain unclear (van Rongen *et al* 2009).

# INTERACTION OF RADIOFREQUENCY FIELDS WITH TISSUES

The electric and magnetic fields produced in the body by a nearby electromagnetic source may cause both thermal and non-thermal biological effects. *Thermal effects* are those caused by the rise in temperature produced by the energy absorbed from oscillating electric fields. The force produced by an electric field

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on charged objects, such as the mobile ions present in the body, causes them to move, resulting in electric currents, and the electrical resistance of the material in which the currents follow the effects in heating. This heat input causes the temperature to rise and it continues to do so until the heat input is balanced by the rate at which it is removed, mostly by blood flowing to and from other parts of the body. It has not yet proved possible to measure these small changes in temperature directly, except those at the outer skin (Adair et al 1999). Theoretical studies up to the present time have restricted themselves exclusively to the computation of specific absorption rate (SAR). The relationship between the SAR and the resulting temperature rise is complex and is significantly dependent on antenna configuration, location and frequency. Non-thermal effects are usually explained by the different ways: the energy quanta of radiation at 0.9 and 1.8 GHz equal 4 and 7 µeV, respectively. Both these values are extremely small and possible changes can arise only if the effect of the electric field within the biological system exposed to RF fields is not masked by thermal noise (i.e., the Brownian motion – which means that all components of biological tissue - ions, molecules and cells - are in constant motion). Another mechanism involving cells concerns the attraction between them in the presence of an electric field (Adair 1994). The electric field polarises the cell, that is to say charges in the cell move so that one side of it becomes positive with respect to the other. The cell is then an electric dipole and attracts similarly polarised cells. For typical cells and frequencies below about 100 MHz, the energies involved are calculated to become comparable to thermal noise in electric fields of E=300 V/m. Other possible biological effects are associated with cell membranes and the movement of currents through the membrane in either direction. Membranes are known to have strongly non-linear electric properties (Montaigne & Pickard 1984). When a voltage is applied across the membrane, the current that flows is not always proportional to the voltage. Part of this nonlinearity may, in fact, be due to the effect of the electric field on the proteins either in the membrane or localized nearby, which assist in the flow of the product currents through the membrane. The membrane also can act as a rectifier.

# HF EMF and free radicals

Recent publications revealed the changes in free radicals generation as a result of high frequency radiation at a frequency of 900 MHz and higher. Some signs of oxidative stress in fetal rat brain (Jing *et al* 2012) and liver (Ozgur *et al* 2010) were described. In experiments with rat lymphocytes, the 930 MHz continuous wave radiation affects the reactive oxygen species formation (Zmyślony *et al* 2004). In experiments with 1800 MHz whole-body radiation in rabbits, the significant increase of oxygen toxicity signs (liver oxidative DNA damage

and lipid peroxidation levels) was revealed (Tomruk *et* al 2010). Also markers of oxidative stress were found in brain samples of guinea pigs after long-term exposure to 890-915 MHz (Meral et al 2007). In the field of the male reproductive system, the experiments with cellular phone radiation suggest oxidative stress damage and a possible way to carcinogenesis (Agarwal et al 2009; De Iuliis et al 2009; Desai et al 2009). More recently, a significant reduction of antioxidant capacity in healthy rats and those with persistent inflammatory state after single 1800 MHz EMF exposure was found when compared with non-irradiated controls (Bodera et al 2013). On the other hand, no significant effect of 900 MHz microwave radiation was evaluated in any experiments performed on murine fibrosarcoma cells (Zeni et al 2007). Also the effect of acute microwave exposure on non-enzymatic antioxidant defense and lipid and protein oxidative damage in the rat frontal cortex and hippocampus was insignificant (Ferreira *et al* 2006). Of course, the methodology used in all the aforementioned experiments was merely indirect (i.e. based on measurement of malondialdehyde, MDA) or using absorption spectrophotometry and the duration of the EMF irradiation was too short for an acute effect.

# **MATERIAL & METHODS**

## <u>Animals</u>

We used 20 mice of both sexes (strain C3H), wild type; 10 for HF EMF exposure, 10 for control. All animals were housed under standard conditions, i.e. 12/12 hours light /dark period (6:00 a.m.-6 :00 p.m) and food and water were available *ad libitum*. All experiments were performed in full agreement with the EU Guidelines for scientific experimentation on animals and with the permission of the Ethical Commission of the Faculty of Medicine in Pilsen.

# HF EMF exposure, SAR measurement

Experimental animals were exposed to HF EMF in a special exposure chamber for small laboratory animals operating at 900 MHz with a Specific Absorption Rate (SAR) measurement; CS patent 21908/2011 (Barcal *et al* 2011). The HF EMF was generated by high-frequency generator (model G4-129, BC Ltd., USSR; 300 MHz – 1,2 GHz) and amplified by EMV amplifier (model 15S1G3, Amplifier Research Corp., USA). SAR measurement was depicted by Power Meter (model 3500, Keithley, USA) placed outside of the chamber and connected with a special matched load.

In our experimental conditions, the average SAR in a mouse (30 g b.w.) was 0.45–1.6 W/kg of the body weight; thus non-thermal effects were expected. Mice were placed into a plastic box located inside the chamber; control mice were kept under analogous conditions without the HF EMF exposure, thus in turn-off mode.

The time duration of experiment was 14 days, 3 hours daily.

#### Examination of free radicals

All animals (exposed and control) were sacrified immediately after the final HF EMF exposure and tissue samples were stored in liquid nitrogen. The samples were then transported to the Institute of Inorganic Chemistry, Academy of Sciences of the Czech Republic (Řež near Prague) where they were tested by EPR (ESR) – Electron paramagnetic (spin) resonance spectroscopy, an analytical and research method for the study of free radicals, excited states, paramagnetic metal complexes, and antioxidants (Rokyta *et al* 2008). The method is based on the absorption of microwave energy by substances with unpaired electron in strong magnetic fields (microwave power: 20 mW, modulation amplitude: 1mT, microwave frequency: 9.3 GHz, temperature 20 °C).

EPR is widely used for the study of short-lived radicals (oxygen radicals, nitroxide radicals), biological samples, photochemical and radiation generation of free radicals as well as other free radicals. In our samples, the presence of hydroxyl radicals OH was measured by spin trapping EPR. The detection of spin adducts and direct measurement of EPR spectra was performed using an E-540 spectrometer (Elexsys, Bruker Biospin, Germany) with online spectra recording and evaluation. Calibration standards  $Mn^{2+}/ZnS$ , and  $Cr^{3+}/MgO$  were used.

### RESULTS

In four tissue samples (brain, liver, kidney and heart) from both exposed and control animals, the level of hydroxyl radicals was measured (*Table 1*). All samples from irradiated animals revealed a statistically significant increase of free radicals production compared with controls (*p*<0.0025; Mann-Whitney nonparametric test – *Figure 1*).

DISCUSSION

Our experiment clearly showed the effect of long-term high-frequency electromagnetic field exposure on free radicals production in four mouse tissue types. Despite these organs representing 3 types of main tissue (epithelial – liver, kidneys, muscular and nervous), the impact of the whole body HF EMF exposure on them was basically almost the same. The results are in accordance with the recent publication describing a significant effect of long-term irradiation (SAR level 0.17–0.37 W/kg) on the proteomics analysis in mice (including neural function related proteins – glial fibrillary acidic protein, GFAP; alpha-synuclein, glia maturation factor beta, GMF; heat shock proteins and



Fig. 1. Values of hydroxyl radicals in four tissue samples of HF EMF exposed animals and controls; error bars indicate the standard error of the mean (SEM) for n=10 independent experiments (in each group).

| CONTROLS  |      |      |      |      |      |      |      |      |      |      |
|-----------|------|------|------|------|------|------|------|------|------|------|
| Mouse No. | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   |
| brain     | 1.57 | 2.77 | 2.21 | 3.7  | 14.7 | 11.5 | 4.7  | 33.3 | 1    | 1.3  |
| liver     | 1.11 | 3.51 | 2.77 | 3.6  | 28   | 15.9 | 10.7 | 17.2 | 4    | 6.4  |
| kidney    | 2.13 | 3.79 | 2.49 | 2.77 | 18.3 | 19.3 | 11.8 | 26.9 | 6.9  | 1    |
| heart     | 1.29 | 2.77 | 4.34 | 2.77 | 11.8 | 16.8 | 16.1 | 15   | 2    | 3.28 |
| EXPOSED   |      |      |      |      |      |      |      |      |      |      |
| Mouse No. | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   |
| brain     | 28.7 | 26.8 | 26.3 | 42.6 | 42   | 43   | 63.5 | 60.3 | 32.4 | 27.1 |
| liver     | 39.8 | 21.3 | 44.4 | 37   | 48.4 | 53.8 | 44.1 | 33.3 | 47   | 28.9 |
| kidney    | 25   | 22   | 31.5 | 41.3 | 58.1 | 57   | 70.1 | 25.8 | 3.1  | 33.9 |
| heart     | 31.5 | 34.2 | 62   | 71.3 | 44.1 | 42   | 40.9 | 34.4 | 29.7 | 32   |

Tab. 1. Hydroxyl radicals concentration in studied samples.

 $/OH/ = table values x 10^{-7} mol.gram^{-1}$ .

cytoskeletal proteins – neurofilaments and tropomodulin). These protein expression changes could be a modulator of brain plasticity alterations, oxidative stress (!) or apoptotic pathway (Fragopoulou *et al* 2012).

Another recent review also referred to further papers dealing with possible role of electromagnetic field (900 and 1800 MHz) in the development of oxidative stress and/or neurodegeneration (Consales et al 2012). More of them revealed the prooxidant effect (Meral et al 2007; Ilhan et al 2004; Ammari et al 2008; Xu et al 2010) than a non-oxidative effect (Irmak et al 2002; Höytö et al 2008) of HF EMF on living tissue. Ilhan et al described marked oxidative damage in rat brain samples exposed to 900 MHz (SAR 2W/kg) during 7 days. Increased levels of nitric oxide (NO), malondialdehyde (MDA), xantine oxidase (XO), and adenosine deaminase (ADA) as well as signs of histopathological changes were detected. A treatment with antioxidant Ginkgo biloba extract, potent free radical scavenger, also decreased significantly oxidative damage in brain tissues. Meral et al (2007) described the effects of 30 days long (12 hours daily) HF EMF exposure (890-915 MHz, SAR 0.95 W/ kg) on brain oxidative stress and some vitamins level in the guinea pig in the measurement of MDA, glutathione (GSH), catalase (CAT) and vitamine A, D<sub>3</sub> and E levels in the brain and blood. A significant increase of MDA and decrease of both glutathione (GSH) and catalase (CAT) levels in the brain without changes in vitamins concentrations were described. The results suggest the influence of HF EMF on the increased lipid peroxidation and free radicals production. In experiments performed by Ammari et al (2008), some changes in the cytochrome oxidase system, specifically the redoxsensitive enzyme and marker of neuronal functional activity were found. The head loop antenna emitting 900 MHz radiation (SAR 1,5 W/kg, 45 min daily or 6 W/kg, 15 min daily, both for 7 days) was used but the significant effect was described only at the higher SAR value. In a recent in vitro study, Xu et al (2010) exposed primary neuronal cultures to HF EMF (1800 MHz, SAR 2 W/kg) for 24 hours. A significant increase of reactive oxygen species (ROS) and also a reduction in the mitichondrial DNA copy numbers was described. A protective effect with melatonin pretreatment, neurohormone with antioxidant capacity, was also found.

On the other hand, an *in vivo* study performed in rabbits (Irmak *et al* 2002) where levels of enzymes (MDA, NO, ADA, XO, SOD, GSH) in brain and blood were evaluated after 900 MHz radiation (2 W peak power, average power density 0.02 mW/cm<sup>2</sup>) 30 min daily for 7 days showed only an increased SOD activity and decreased NO levels in the blood. No further change in any brain samples was found. Also an exposure of the human neuroblastoma cell line (SH-SY5Y) and mouse L929 fibroblast cells to HF EMF (872 MHz, SAR of 5 W/kg for 1 hour) showed no effect on GSH levels and any DNA fragmentation were found. (Höytö *et al* 2008).

The results of some experiments, especially from different brain areas, suggest a free radicals (ROS) overproduction as one of the possible pathogenic mechanisms. There are also papers in the literature about the effects of RF fields on isolated nerve cells (neurons), on cultured nervous tissue, on living brain slices, on brain function in experimental animals, on the blood-brain barrier and finally on the behavioural measures of brain function (Repacholi 1998a; Repacholi 1998b; Liu & Cleary 1995; Hermann & Hossmann 1997). In experiments performed by Salford et al (1994), 915 MHz electromagnetic field (pulse modulated) had a significant effect on the permeability of blood-brain barrier and, also neuronal damage in the brain cortex, hippocampus and basal ganglia in exposed rats was described (Salford et al 2003). In the another recent study (Mausset-Bonnefont et al 2004), short-term acute exposure (15 min) to 900 MHz induced a strong glial reaction in the brain.

Results from 9 epidemiological studies (United States, Sweden, Denmark, Finland, Germany) suggest a relative risk of exposure to between 1.3 and 4.6 GHz for acoustic neuroma and uveal melanoma (Kundi 2004). Also the evidence for enhanced cancer risk with increasing latency and duration of mobile phone use was depicted, but all studies have some methodological deficiences, for example a too short duration or the different determinations of exposure.

The behaviour of animals can be a very sensitive indicator of adverse health consequences. Early signs of potential insult are often behavioural rather than anatomical (Salzinger 1994). Behavioural experiments on animals are used to investigate the biological basis of memory, and studies with non-human primates can serve as a model of human cognitive functions. For example, the whole-body exposure to 2.45 GHz EMF (45 min before behavioral testing) did not alter the anxiety response in elevated plus maze despite the previously found increase in the number of benzodiazepine receptors in the rat cortex due to EMF irradiation (Cosquer *et al* 2005).

Results concerning whole-body exposure of 900 MHz (power density max. 10 W) in our laboratory showed only gentle changes of excitability after acute and long-term exposure using audiogenic epilepsy method in mice; yet also changes of the CNS inhibition using the pentobarbital sleep method suggested a lower mortality in HF EMF exposed animals (Cendelín et al 2004). Also spatial learning ability in mice after exposure to HF EMF (tested in Morris water maze) revealed only weak differences (some of them significant) dependent on the strain and age (Cendelín et al 2001; Voller et al 2003). Nevertheless, we described some somatic and neuro-behavioral conesequences of chronic HF EMF exposure in healthy and partly neurodefective mice. There HF EMF irradiated Lurcher mutant mice, which represent an animal model of olivocerebellar degeneration, exhibited a significantly lower swimming speed in the Morris water maze compared with non-irradiated

controls (Vožeh et al 2007). Further, a histochemical examination showed a lower NADPH-diaphorase positivity (marker of NO production) in the hippocampal dentate gyrus after chronic HF EMF exposure which was more evident in Lurcher mice when compared with controls including strain differencies (Vožeh et al 2003). The immunocytochemical depiction of *c-fos* (as acute cellular stress marker) in hippocampus revealed differences between irradiated and control animals; a clear c-fos activity was evident only in animals after HF EMF exposure (Jelínková et al 2003). Likewise an evaluation of spontaneous brain cortical activity (ECoG) recorded simultaneously with HF EMF exposure suggested changes in brain electrogeny. ECoG revealed a shift towards lower frequencies of waves. Spontaneous hippocampal activity showed the opposite changes with an increase of theta oscilations during HF EMF exposure (Barcal et al 2005).

In conclusion, we can conclude that these experiments, which complement our previous mainly functional ones, show the effect of long-term irradiation of high-frequency electromagnetic field (900 MHz) on free radicals production in four mouse organs. A highly sophisticated spin trapping (EPR) method, probably for the first time used for hydroxyl radicals detection in such biological samples, confirmes the positive effect of the whole body chronic HF EMF irradiation on oxidative stress in specimens examined. Because the finding is consistent with results of series of experimental works from the last time, our evidence further supports the hypothesis that in certain situations it is necessary to take HF EMF as a possible pathogenetic factor.

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#### **Conflict of Interest Statement**

The authors declare no conflicts of interest in this work.

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