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Research Article

Deleterious Effects of Exposure to 900 MHz Radiofrequency Waves on Brain Trace Elements Improved by Administration of Vitamins

Mansour Azimzadeh¹⁰, Gholamali Jelodar^{1*10}

¹Department of Basic Science, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

Abstract

Background: There is an increasing public concern about the harmful effect of exposure to electromagnetic radiation emitted from base transceiver stations (BTS).

Objectives: This study was carried out to evaluate the impact of exposure to 900 MHz radiofrequency (RF) on the concentration of brain trace elements (TEs), including zinc (Zn), manganese (Mn), copper (Cu), and iron (Fe), as well as the protective effect of vitamins (C and E) supplementation.

Methods: In this study, 30 male rats were assigned into three non-exposed groups, including sham I, II, and III receiving saline, vitamin C, and vitamins C+E, respectively, and three exposed groups, including sham-exposed (saline), test I (vitamin C), and test II (vitamins C+E). The exposed groups were exposed to RF for one month (4 h/day) and the brain TE levels were measured using atomic absorption spectrophotometry. **Results:** Compared with the non-exposed groups (I, II, and III), a remarkable elevation in the concentration of Mn, Fe, Cu, and Cu/Zn ratio accompanied by a significantly lower level of Zn was noticed in the rats' brain of the sham-exposed group (P < 0.05). However, pretreatment with both vitamins C and C+E improved the evaluated TE imbalances caused by exposure to 900 MHz RF with no significant difference between the two test groups (P > 0.05).

Conclusion: Disruption of brain TEs homeostasis caused by the exposure to 900 MHz RF improved and normalized by pretreatment with vitamins C and E+C.

Keywords: Electromagnetic wave, Copper, Iron, Manganese, Vitamins C and E, Zinc

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Background

Mobile phones and the base transceiver stations (BTS) antennas are the most popular radiofrequency wave (RFW) user devices, and there is increasing public concern about their potential adverse health effects. Several studies have investigated and reported the detrimental effects of radiofrequency (RF) on various animal tissues (1, 2).

In fact, due to the position of mobile phones during a voice call, and relatively high RFW absorption in the brain tissue, clinical and experimental studies revealed the potentially deleterious effects of these waves on tiredness, anxiety, headache, stress (3), and impairment of learning processes (4, 5).

Trace elements (TEs) have a critical functional role in various physiological and biochemical actions in the biological systems in humans and animals (6). Zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) have a protective function against the generation of reactive oxygen species (ROS) in the brain and neurodegenerative diseases, and they regulate enzyme activation and gene expression (7). Fe has a main role in oxidative energy production and is involved in respiratory chain proteins and oxidative enzymes (8). Cu has a functional role in enzymatic activities linked to brain signal transduction and neurotransmitter function (9) and is presented in the enzymes and proteins, including Cu/Zn superoxide dismutase and cytochrome C oxidase (10). Furthermore, Fe and Cu are well-known redox-active metals with a functional role in the reactive radicals generation and cycling reactions, which can develop uncontrolled oxidative stress (11).

Alteration of the brain Zn homeostasis causes neurological and behavioral disorders such as Alzheimer's disease and impairment of cognitive and learning processes (12). Besides, both Cu and Zn have a critical duty in the free-radical generation chain and decreasing the peroxidation ratio (13). The Cu/Zn ratio is a wellknown sensitive indicator of inflammation and oxidative stress (14).

Mn has a vital role in brain development and function, carbohydrate metabolic process, and maybe enzymatic antioxidant defenses (MnSOD, and catalase) (15). Mn has a well-known neurotoxic property and maybe the central mechanism through which oxidative stress develops degenerative brain disorders (16).

*Correspondence to

Email: jelodar@shirazu.ac.ir

Gholamali Ielodar.



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An endogenous redox agent, vitamin C, is arguably a potent water-soluble chain-breaking antioxidant, along with lipid-soluble vitamin E that limits the generation of ROS, especially in the brain, and protects membrane lipids and proteins from oxidative damage. Vitamin C is a cofactor for several enzymes and acts as an electron donor. The reduced activity of vitamin C play an important role in iron and folic acid metabolism, regenerating vitamin E, and scavenging ROS (17).

Both vitamins E & C have a neuroprotective function and are known as necessary molecules in neuronal survival and maintenance in the central nervous system (CNS), particularly against oxidative stress-linked diseases (18, 19). Moreover, the protective effects of vitamin C against RFW on learning and memory (4, 20) and oxidative index (21) have already been reported.

Objectives

In the experimental studies about the evaluation of electromagnetic side effects, several parameters, including specific absorption rate (SAR), duration of exposure, the frequency of waves, and distance from radiation source have been identified as the most important determining factors. The main differences between our experimental condition and others include the frequency wave (900 MHz), SAR (0.035 W/kg), and duration of exposure (4 weeks). Moreover, most of the studies used serum/plasma but we used brain tissues because the brain is the most sensitive organ for electromagnetic side effects due to the high content of lipid and the presence of the blood-brain barrier. In addition, we used the vitamin treatment (C and C+E) as an antioxidant and neuroprotective factor with considering the functional and vital role of these vitamins on the brain TEs homeostasis.

Materials and Methods Animals

In this experiment, 30 colony-bred male rats (Sprague Dawley) weighing 190 ± 20 g in the Shiraz Animal House Center were included. Animals were kept at a constant temperature (20 ± 2 °C), 12-h light-dark cycle, and had free access to water and food. Exposure time was set and carried out between 9 am and 1 pm.

Experimental Protocol

We studied the effect of 900 MHz RF on the Cu, Fe, Mn, and Zn concentrations in the brain tissue and the protective role of pretreatment of vitamins (C or E+C) after 30 consecutive days of treatments. All rats were divided into six equal groups (n = 5) as the three nonexposed groups, including sham I (saline), sham II vitamin C (250 mg/kg), sham III vitamin C (200 mg/ kg + E 100 mg/kg), and three exposed groups (exposed for 30 continuous days, 4 h/d), including sham-exposed (saline), test I (vitamin C 250 mg/kg) and test II (vitamin C 200 mg/kg + vitamin E 100 mg/kg). Vitamins and saline were administrated orally by gavage.

Radiofrequency Field Exposure Device

We used a simulator emitting a 900 MHz field through a vertical antenna (12 cm) which produces a power density of 0.6789 mW/cm² measured by a spectrum analyzer (Rohde and Schwarz, Germany). The signal generator was positioned at a 1-meter distance from the cages of exposed groups (groups 4-6) and the value of specific absorption rate (SAR) was detected by a field-probe device (300 kHz - 18 GHz, Wave Control, Spain) at 0.035 W/kg.

Sampling and Tissue Preparation

On the last day of the exposure period, the rats were anesthetized using a 2% diethyl ether-saturated cotton ball in a chamber for 3–5 minutes and euthanized by whole blood collection through the heart puncture. The brain tissue was instantly harvested and kept at -70 °C for evaluating TEs (Cu, Fe, Mn, and Zn) following washing with distilled water.

Evaluation of TEs

Atomic absorption spectrophotometry (Shimadzu AA-670, Kyoto, Japan) was used to detect the amount of Cu, Fe, Mn, and Zn. The UV-Visible detector was used for Cu: 324.8 nm, Fe: 248.3 nm, Mn: 279.6 nm, and Zn: 213.9 nm. To prepare brain tissues for evaluation of the TEs, they were dried at 60°C for 24 hours, dissolved in a blend of nitric acid and perchloric (7:3 ratios, respectively), then kept in a 55°C water bath to complete digestion and double distilled water was used to dilute the samples.

Statistical Analysis

All collected data were expressed as mean \pm SE. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. *P* value was set at < .05.

Results

The concentrations of Cu, Fe, Mn, and Zn levels (mean \pm SEM) and Cu/Zn in the brain tissue are presented in Figure 1.

In the sham-exposed group, the mean Zn level (12.29 ± 0.63) was significantly lower than other study groups, while a significant increase in the mean Cu $(1.27\pm.16)$, Fe (29.48 ± 1.69) , Mn (0.49 ± 0.01) , and Cu/Zn ratio (0.26 ± 0.01) was noticed.

As depicted in Figure 1, vitamin supplementation (test groups I & II) significantly improved all TEs status in the brain tissue, but there was no significant difference between test I (vitamin C) and test II (combined C+E) groups.

A significant increase was noticed in the Fe level in the test II group compared to the sham III group (P < 0.05). The Zn level significantly decreased in the test I group compared to the sham I and II groups (P < 0.05). There



Figure 1. The concentration of all trace elements was measured by using atomic absorption spectrophotometry. Columns represent mean \pm SEM. Sham groups (I, II & III) were not exposed to RFW and received distilled water, vitamin C 250 mg/kg, or a combination of vitamins E (100 mg/kg) and C (200 mg/kg), respectively. Sham-exposed, Test I, and II groups were exposed to 900 MHz RFW (4 h/day for 30 continuous days) and received distilled water, vitamin C (250 mg/kg), or vitamins E (100 mg/kg + C 200 mg/kg), respectively. (+) The significant difference with all groups. (++) The significant difference with sham III. (#+) The significant difference with sham III. (##) The significant difference with test I (*P*<0.05). Abbreviations: Cu, copper; Fe, iron; Mn, manganese; Zn, zinc.

was a significant decrease in the Mn level in sham III group compared to the sham I group. Cu/Zn ratio in the test I group was significantly higher than sham II (P < 0.05).

Discussion

The findings of this study indicated that exposure to 900 MHz RF can cause imbalances in the brain TEs homeostasis. In agreement with our results, it has been reported that exposure to 950 MHz (1 h/d, 8 weeks) disrupted brain TEs homeostasis (22). Another study reported a decrease in Zn level following exposure to RFW emitted from the mobile phone (1800 MHz) (23). Moreover, some previous reports indicated that mobile phones and Wi-Fi irradiation induces oxidative damage in the kidney and testis by decreasing glutathione, Cu, and total antioxidants, while augmenting the rate of lipidinduced peroxidation and the Fe level (24).

However, there is controversy in the reported results following exposure to different electromagnetic fields (EMF). There was no alteration in the plasma content of Cu, Fe, Zn, and Mn in dairy cows exposed to 60 Hz extremely low-frequency (ELF) EMFs (25). Exposure to ELF-EMF (50 Hz) did not alter the serum concentration of Cu, Zn, and Fe in rats (26). Different methodologies, including the duration of exposure, the frequency used, distance, and intensity can be the reasons for discrepancies in different experiments.

A growing body of data has a consensus on oxidative stress as the important mediator of exposure to the

radiation of mobile phones (27, 28). On the other hand, some important properties in the brain tissue such as fairly weak enzymatic defense, higher lipid density, Fe, and high aerobic metabolism are prone to lipid-induced peroxidation and finally oxidative stress (29).

A high level of Mn in the brain tissue can be neurotoxic, while a low level is required for proper and normal functioning (30). In our study, the Mn level in brain increased following exposure to 900 MHz RF, which is in consonance with other reports (31). Moreover, the accumulation of Mn in the liver, kidney, and brain following exposure to the EMF has already been reported (32). Mn uptakes into the brain either via the bloodbrain barrier (BBB) or cerebrospinal fluid (33) and slow turnover of Mn in the brain (32) have been proposed for possible mechanisms.

In this study, pretreatment with both vitamins (C & C+ E) significantly improved the levels of all the evaluated brain TEs (Cu, Fe, Mn, Zn, and Cu/Zn ratio) following exposure to RF. As mentioned, oxidative stress is the primary cause of the deleterious effects of RFWs. Moreover, vitamins C and E have vital and interesting properties such as long-lasting defense against Fe-induced oxidative stress and Mn-induced hydroperoxides and neurotoxicity (34, 35), as well as neuroprotective property against neurodegenerative diseases (36). Besides, the beneficial effects of both vitamins against RF side effects on different body organs including the brain have been reported (4, 21). Moreover, combined treatment of vitamins C and E improves learning and memory and cognitive function (4, 37) in scopolamine-induced dementia (38).

The BBB is one of the regulators of the TEs balance in the brain (39), and some previous studies have suggested an increase in permeability of the BBB following exposure to mobile phone radiation (40, 41).

Accordingly, two main possible pathways, including oxidative stress and increased BBB permeability, can be responsible for the disturbed homeostasis of TEs in the brain following exposure to 900 MHz RF.

Conclusion

Disturbed brain TEs (Cu, Fe, Mn, and Zn) were the outcome of exposure to 900 MHz RF. Pretreatment with both vitamins (C & E+C), possibly due to their antioxidant and neuroprotective properties, ameliorated and normalized the negative and disruptive effects of exposure to RFW. There was no significant difference between the pretreatment with vitamin C alone or in combination with vitamin E. The finding of this study provided evidence about the beneficial effects of exposure to RFW in an experimental condition which may be applicable in humans. However, further studies are needed to confirm the findings of this study.

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Conflict of interest

The authors announce to have no conflicts of interest.

Ethical Approval

The Institutional Research Ethics Committee of the School of Veterinary Medicine of Shiraz University approved all of the investigations involving animal use and care.

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