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Original Article

Protective effects of melatonin and omega-3 on the hippocampus and the cerebellum of adult Wistar albino rats exposed to electromagnetic fields

Gamze Altun[a,*], Suleyman Kaplan[a], Omur Gulsum Deniz[a], Suleyman Emre Kocacan[b], Sinan Canan[c], Devra Davis[d,e], Cafer Marangoz[f]

[a] Department of Histology and Embryology, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey
[b] Department of Physiology, Medical Faculty, Ondokuz Mayıs University, Samsun, Turkey
[c] Department of Psychology, Üsküdar University, Istanbul, Turkey
[d] Department of Medicine and Public Health, The Hebrew University, Jerusalem, Israel
[e] Environmental Health Trust, Teton Village, WY, USA
[f] Department of Physiology, Medical Faculty, Medipol University, Istanbul, Turkey

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ABSTRACT

The purpose of the study was to investigate the effects of pulsed digital electromagnetic radiation emitted by mobile phones on the central nervous system of the adult Wistar albino rats. The study evaluated structural and functional impacts of four treatment arms: electromagnetic field (EMF) exposed; EMF exposed + melatonin treated group (EMF + Mel); EMF exposed + omega-3 (ω3) treated group (EMF + ω3); and control group (Cont). The 12-weeks-old rats were exposed to 900 MHz EMF for 60 min/day (4:00–5:00 p.m.) for 15 days. Stereological, biochemical and electrophysiological techniques were applied to evaluate protective effects of Mel and ω3. Significant cell loss in the CA1 and CA2 regions of hippocampus were observed in the EMF compared to other groups ($p < 0.01$). In the CA3 region of the EMF + ω3, a significant cell increase was found compared to other groups ($p < 0.01$). Granular cell loss was observed in the dentate gyrus of the EMF compared to the Cont ($p < 0.01$). EMF + ω3 has more granular cells in the cerebellum than the Cont, EMF + Mel ($p < 0.01$). Significant Purkinje cell loss was found in the cerebellum of EMF group compared to the other ($p < 0.01$). EMF + Mel and EMF + ω3 showed the same protection compared to the Cont ($p > 0.05$). The passive avoidance test showed that entrance latency into the dark compartment was significantly shorter in the EMF ($p < 0.05$). Additionally, EMF had a higher serum enzyme activity than the other groups ($p < 0.01$). In conclusion, our analyses confirm that EMF may lead to cellular damage in the hippocampus and the cerebellum, and that Mel and ω3 may have neuroprotective effects.

1. Introduction

Mobile phones were first introduced into the market in the 1970s with no pre-market safety testing [1]. As most users were adult male medical and military personnel, the average call was assumed to last six minutes and was judged safe if it resulted in no increase in temperature from the weak non-ionizing radiation emitted. Today, very young persons or those with much smaller brains and bodies in comparison to the 175 cm tall, 88 kg male phantom against which phones were first tested for their capacity to change temperature, use more than half of the world's nearly 7 billion phones. Because they are often held directly next to the head and pulsed digital radiation is absorbed about 2 cm into the brain, the effects of mobile phones particularly on the central nervous system may be remarkable. Biological agents can be thought of as having two distinct types of impacts: those that constitute direct physical impacts such as change in structure, cell count, proteomics, etc.; and those that constitute functional or behavioral effects such as change in response time, memory, or other standardized measure of performance that have been well characterized through a battery of tests [2–4].

Previous investigations have confirmed a number of important structural impacts of EMF. A decrease in the number of Purkinje cells in the rats exposed to a 900 MHz electromagnetic field (EMF)

* Corresponding author.
E-mail address: gamzeyayla.omu@gmail.com (G. Altun).

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was previously reported [5]. Additionally, a significant decrease in the number of granular and pyramidal cells of the hippocampus, which play a key role in memory-related functions, was shown by stereological techniques that allow 3-dimensional imaging of location [5–7]. Other destructive effects have also been determined by different methods. Damage to the cortex, cerebellum, hippocampus, and basal ganglia have resulted from neuronal damage induced by exposure to a 900 MHz EMF [8].

Other studies have demonstrated critical functional changes after EMF exposures. The effect of EMF exposure on increasing permeability of the blood-brain barrier (BBB) has been well documented for more than four decades [9,10]. This increased permeability affects homeostasis and may cause leakage of serum albumin into brain tissue. This process also leads to neuronal degeneration in the brain [11]. Currently, this property of EMF/RF is being used for enhanced uptake of chemotherapy in the treatment of brain cancer, while amplitude modulated current is being employed as part of a treatment involving tumor-treating fields (TTF) that can interfere with post-mitotic spindle formation and increase survival of seriously ill brain and pancreas cancer patients [12,13].

Exogenous influences such as extra-low-frequency electromagnetic field (EMF) have been shown to effect pain and inflammation by modulating G-protein receptors, down-regulating cyclooxygenase-2 activity, and affecting the calcium/calmodulin/nitric oxide pathway. Investigators have reported changes in opioid receptors and second messengers, such as cyclic adenosine monophosphate (cAMP), in opiate tolerance and dependence by showing how repeated exposure to morphine decreases adenylate cyclase activity causing cAMP to return to control levels in the tolerant state, and increase above control levels during withdrawal [14].

In addition, other functional impacts of EMF exposure may affect neuronal membranes and organelles, such as lysosomes and mitochondria; consequently, heavy metals and reactive oxygen species (ROS) may reach high levels in intracellular areas [15,16]. Thus, electromagnetic radiation-induced tissue damage may be associated with ROS. In this context, one of the several molecular pathways involved in EMF exposure-induced neuronal damage is the caspase3-dependent pathway [17]. The extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) pathway also plays a crucial role in signal transduction regarding cell growth and differentiation [18,19]. Friedman et al. have effectively shown that MAP-kinase are also affected by low levels of EMF/RF and thus electromagnetic radiation emitted from mobile phones may induce the ERK cascade and the other cellular processes [20].

Neuronal ultrastructural effects of EMF exposure on these pathways have been investigated using proper immunohistochemical and electron microscopic analyses in a study conducted by Tang et al. [21]. The hippocampus and parietal cortex revealed tight and regular formation of neurons with neuroglia cells in the non-exposed group. Electron microscopic analysis in the group exposed to 900 MHz EMF for 28 days showed many empty and inadequate areas surrounded by vessels and neurons [22]. Moreover, this is consistent with many studies showing the harmful effects of EMF on the developing nervous system during prenatal and postnatal periods [23]. In particular, EMF exposure can lead to neuronal death and inhibits transformation of neuronal stem cells into neurons [23]. The structural damage has produced many functional disorders, such as cognitive impairment [5–7,22]. Oxidative stress is a type of disequilibrium between antioxidants and free radicals. After electromagnetic radiation (EMR), the presence of increased free radicals causes the deterioration of membrane integrity by acting on proteins and nucleic acids. This also leads to some gene mutations and antioxidant defenses, which occur to counter these effects [24,25]. Measurement of ROS to determine these effects is very difficult due to their high reactivity, short half-life, and high concentrations. The intracellular damage activates superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) [26,27]. The first enzymatic defense against to the ROS is SOD [28,29]. CAT produces a peroxidative reaction in the presence of low levels of H_2O_2 and high concentrations of free electron donors [30]. Normally, the main enzyme responsible for the detoxification of H_2O_2 is GSH-Px, and it inhibits lipid peroxidation. GSH-Px produces H_2O_2 from water by reducing GSH [28,29]. By means of this reaction, GSH becomes oxidized glutathione (GSSG) [31].

Many studies have examined both the neuroprotective effects of some antioxidants as well as the damaging impacts of EMF. In term of this point, the efficiency of melatonin (Mel) and omega-3 (ω3) to induce repair or otherwise interfere with the damaging effects of EMF is noteworthy. Research shows that Mel is an antioxidant agent, as well as ω3 [32]. As a free radical scavenger, Mel has a high degree of lipophilicity and therefore does not require any binding receptor for its effects [33–35]. Additionally, ω3 polyunsaturated fatty acids (ω3 PUFAs) are an essential type of fatty acids and play a crucial role in neurological function because they are integral membrane components and energy substrates [36].

A few studies have been done using stereological techniques to evaluate the effect of EMF exposure on the CNS. In addition, structural changes after prenatal exposures to a 900 MHz EMF have been investigated by several studies [5,6]. However, there are no enough studies regarding evaluation of EMF exposure on the cellular number in the brain and cerebellum of the rats using quantitative techniques. In this context, the present study was designed to investigate the structural and functional impacts of EMF radiation on the brain. In addition, the possible neuroprotective effects of Mel and ω3 have also been searched against EMF exposure.

2. Material and methods

The main purpose of this study was to investigate the effects of 900 MHz EMF exposure and the possible neuroprotective effects of the Mel and ω3 on the granular and pyramidal cells in the hippocampus and Purkinje and granular cells in the cerebellum of adult rats. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two important fatty acids that can be found in fish oil [37], and for this reason fish oil is an important nutritional source. To obtain reliable data for EMF exposure and the neuroprotective ability of these substances, the optical fractionator method – which is a type of unbiased stereological probing technique – paired with cognitive tests and biochemical parameters of oxidative stress. Electrophysiological analysis may inform us about sensitive alterations of interactions in the brain. In this context, electrophysiological assessment was also used in the present study.

We employed two overall methods to evaluate both structural and functional impacts on the young animal brain and their subsequent performance [16,38,39]. Prior to sacrifice, animal behaviors were evaluated by the passive avoidance test [16], where animals were trained to avoid a noxious stimulant such as an electric shock for a food reward. In this behavioral test, three parts were conducted, including an exploration test (1), a learning test (2) and a retention test (3). The exploration test was conducted in three trials. The rats were kept in the center of the light compartment and the door was kept open for 3 min. The total time to enter the dark compartment was noted for each trial. When the rats entered the dark compartment, the door between the compartments was closed and 0.5 mA electric shock was given for 3 s. Then, the ceiling was opened and the rats were returned to the home cage. Each rat received retention tests that were applied after the 24 and 48 h of 0.5 mA electric shock. During this period of rest, the sliding door was open.

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