Effects of cell phone radiation on migration of granule cells in rat cerebellum

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Abstract

Introduction: There are a growing number of cell phone devices and they are one of the most popular sources for electromagnetic waves. However, there are many concerns regarding their biological effects on the body, especially the central nervous system. Therefore, the aim of this study was to evaluate the effects of cell phone radiation on histology of the cerebellar granular cells in the neonatal rat.
Materials and methods: We selected some Wistar rats. Then, we randomly divided their newborns into four groups. One of the groups was control and the other three were experimental groups. Groups I, II, and III were exposed to radiation from cell phone (with a distance of 10 cm from the rats) for a duration of 30 minutes, 2 hoursand 8 hours, respectively. After killing of the rats and preparing histological sections, we counted the granular cells of the cerebellum using Motic software. We measured the thickness of granular layers with Nickon software. Then, after data collection, statistical analysis was conducted using Tukey and Anova tests.
Results: Results showed that cell population in internal granular region significantly decreased in group III. Also, results indicated a reduction in the external granular layer thickness in group III. Furthermore, cerebellar weight to body weight ratio in group III was significantly reduced as compared with the other groups.
Conclusion: Results of this study showed that cell phone electromagnetic radiation can reduce the number of internal granular cells of the cerebellum and the external granular layer thickness is reduced.

Keywords: Granular Cells, Cerebellum, Cell Phone Radiation

Introduction

Life on earth equals being immersed in a sea of natural electromagnetic fields. The electromagnetic spectrum has a very broad frequency range. These include radiofrequencies, microwaves, visible light, infrared andultraviolet radiation, X-ray, and gamma rays. Waves emitted from cell phones are also part of the electromagnetic waves. They exist in the frequency range of 900 MHz to 1 GHz and in the wavelengths of 1 mm to 1 m in the electromagnetic spectrum. (1) Nowadays, cell phones are one of the most common devices emitting electromagnetic waves.

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Almost half the world's people have access to cell phones at different ages. Expansion of mobile network and the variety of services offered by their network service providers such as short messaging service, sending pictures, videos, easy and quick electronic payments, increase public interest to the use of these devices. Consequently, cell phone is not a luxury device anymore and it is one of the essential tools. (2) With the increasing number of cell phone users and numerous reports in recent years about harmful effects of radiation on developmental processes in various organs of the human body, serious concern has been created. Despite guarantees of the board of industrial offices including radiological Protection Board in UK, there are still many doubts on the adverse effects of such devices (3). Effects of electromagnetic fields on biological systems have been studied fairly extensively. However, due to the lack of conclusive results and unclear action mechanisms for these fields, investigation in this area continues to remain active (4). Some adverse effects of EMF include: impact on reproductive parameters (5), damage to brain cells DNA (6), Induction of apoptosis in brain tissue (7-9), reduction in the number of pyramidal neurons and increasing the number of ischemic neurons in the cerebral cortex (10), disruption of brain and behavior activity (11, 12), increased permeability of the blood - brain barrier (13), induction of oxidative stress by increasing free radicals, increase in the lipid peroxidation and changes in tissue antioxidant defense system (14, 15), decline in learning and memory (16, 17), induction of brain tumors, headaches, depression, hot regions around the ears, and impaired attention (18, 19). On the other hand, cell migration is a crucial process for the organization of the evolving nervous system. On the other hand, even though the structure of the brain at birth is complete, the degree of plasticity continues throughout life including axonal remodeling, synaptogenesis, production of new neurons and their migration (20). This change is also true for the cerebellum and any disruption in neuronal migration can cause structural abnormalities including Lissencephaly, pachygria, cognitive dysfunction and motor deficits in the interpretation of the data (21). Cerebellar granule cells are among the most numbered cells in the cerebellum. Migration of these cells occurs in a dynamic and rapid fashion. Their mechanism of action is largely unknown, but it has been demonstrated that factors such as alcohol affects the intracellular calcium and can reduce the migration of these cells (22, 23). Despite reports on the effects of electromagnetic radiation in reducing the number of cerebellar Purkinje cells, there exist morphological changes in cellular organelles and abnormal electrophysiological activity (24, 25). However, no report has been released on the effects of the waves on histological and ultrastructure of granular cells in the cerebellum during development. Therefore, in this study we examined the effects of cell phone radiation on the migration of granule cells during the development of the rat cerebellum.

Materials and methods

This study was conducted on Wistar rats aged 6-8 weeks and weighing 200 ± 0.53gr, obtained from Pasteur Institute, Iran. First, they were kept in standard conditions of the animal house for one week including temperature of 23 to 25 ° C, humidity of 50 to 55 percent and light cycle of 12 hours. They were kept inside a plastic cage (to avoid any nuisance electromagnetic waves). Then, we put two separate cages including 2 female mice and 1 male mouse in each one for overnight mating. After vaginal plug, they were separated and placed in the same conditions. After delivery, we separated the newborns into 4 groups (n = 10) including one control and three

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experimental groups. The experimental groups were divided into 3 groups as follows:

Group I (n = 10): included rats that were exposed to cell phone radiation for 30 minutes daily on days 7 to 13 of pregnancy(G1).

Group II (n = 10): included rats that were exposed to cell phone radiation for 2 hours daily on days 7 to 13of pregnancy(G2).

Group III (n = 10): included rats that were exposed to cell phone radiation for 8 hours daily on days 7 to 13of pregnancy(G3).

We used cell phones (Nokia brand, made in Finland) on conversation mode, for radiation. Since previous studies showed that the greatest impact of cell phone radiation were in the range of 900 to 1800 MHz and at a distance of 10 cm from the body (26), we used our cell phones placed 10 cm apart from the animal. On the fourteenth day after birth, the animals were anesthetized by chloroform. In all groups, body weights of newborn rats were measured before necropsy with a digital scale. After killing the animal, using a scalpel blade or scissors, we made a longitudinal incision along the sagittal suture and a transverse slot along the coronal suture in the base of their skull. After removing the skull wall and a transverse incision in the medulla oblongata, skull was separated from the spinal cord and the cerebellum as a whole was removed from the cranial cavity with precision. The cerebellar weight was measured in all neonates with a digital scale. Then ratio of cerebellum weight to body weight was calculated based on the percentage and were studied using statistical tests. Then, the cerebellum was fixed in 10% formalin, and tissue preparations were made using routine histological procedures. To examine and count the granular cell, 6 micron sagittal serial sections from the cerebellar vermis were prepared. Among sections, only those cuts which were close to the midline vermis and were not visible in the cerebellar nuclei were selected and stained with Hematoxylin-Eosin (H & E) and Violet. Results were imaged using an optical microscope and cells were counted using the Motic software. Also for measuring the thickness of the granular layer, taken photos were transferred to a computer and were measured using Nikon Digital software (Sight-DS-L2 model). For statistical analysis, Tukey, Kruskal-Wallis and analysis of variance (ANOVA) were used showing a significant difference ($P < 0.05$).

Results

Evaluation of cerebellar tissue sections by light microscopy and Motic software showed a significant decrease in the internal granular cell population and granule cells in the molecular area (Figure 1).

Also, using a graduated eyepiece and a magnification of 10, thickness of cerebellum granular layer was studied in different groups. Results showed a significant reduction in the thickness of the external granular layer in group III (exposure for 8 hours per day) compare to other groups ($P < 0.05$). This value was 50±54.6 mm in the control group, 50±52.5 mmin the 30 minutes exposure group, in the 2 hours exposure group 49±17.2 mm, and 40±04.8 micrometerin the 8 hours exposure group. Yet, reduction in the external cerebellar granular layer thickness in groups I and II was not significantwith respect to each other and relative to the control group (Figure 2). As concluded in this research, the cerebellar weight in the newborns exposed to cell phone radiation for 8 hours on days 7 to 13 of pregnancy decreased significantly in relation to their body weight, compared to that of the control group as well as I and II ($P<0.05$)(Table1).

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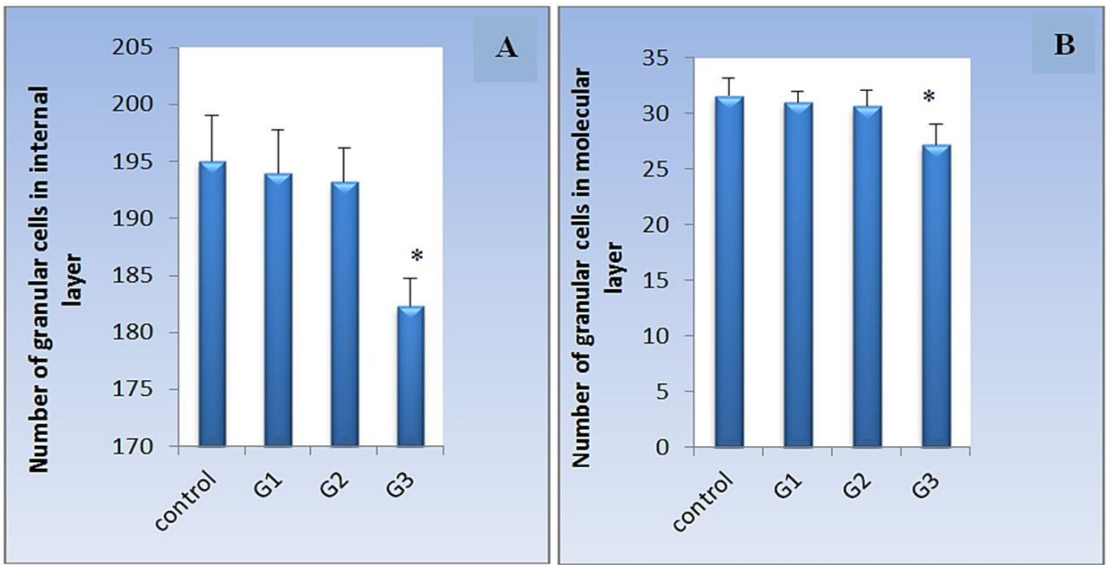

Figure 2.Diagrams shows the loss of granular cell populations at different exposure times in the internal granular layer (A) and molecular layer (B). Exposureto cell phone radiation from days 7 to 13 of pregnancy for 8 hours a day (G3) significantly decrease the number of granular cells in both layers.

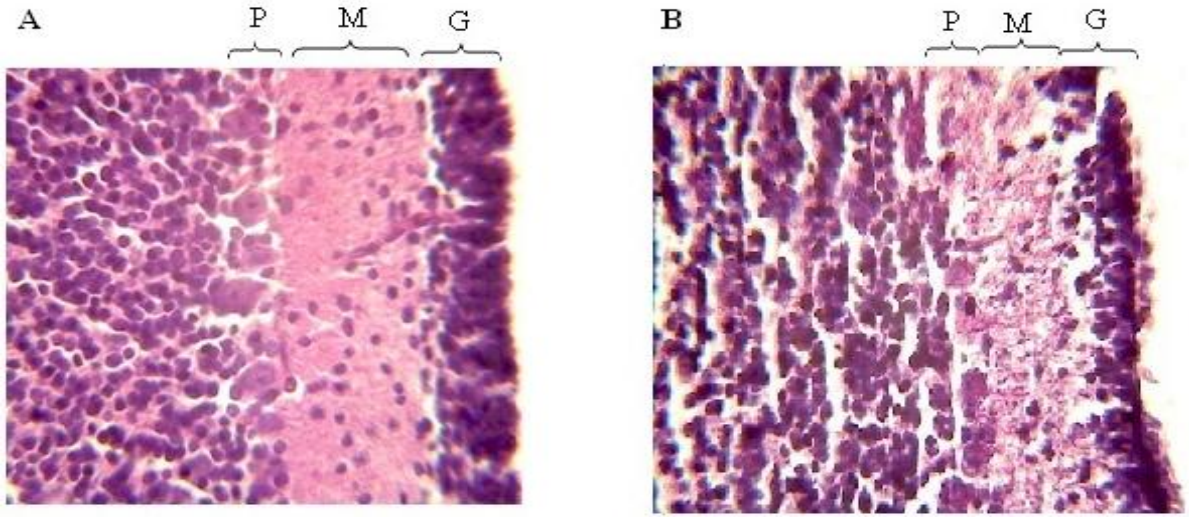

Figure 2.Optic microscope image of cerebellum of the newborns before (A) and after exposure to cell phone radiation (B) from days 7 to 13 for 8 hours a day. The image shows a decrease in the thickness of the external granular layer (G) (Arrows), molecular layer (M), and Purkinje cell layer (P). (Stained with H &E; ×60).

Table 1.Mean body weight, cerebellar weight (in grams) and mean diameter of the external granular layer (micrometers) in Wistar newborn rats.

Variables	Control group	Experimental group I	Experimental group II	Experimental group III
Body weight	15.13 ± 0.25	15.14 ± 0.86	15.19 ± 0.41	19.84 ± 0.43
Cerebellar weight	0.98 ± 0.73	0.97 ± 0.68	0.95 ± 0.33	0.79 ± 0.15
Mean diameter of the external granular layer	50 ± 54.6	50 ± 52.5	49 ± 17.2	40 ± 04.8

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Discussion

Results of this study showed that cell phone RF waves in the post-natal period for 8 hours reduces the number of granular cells, reduces the thickness of the granular layer of the cerebellum and ultimately cause reduced cerebellar weight to body weight ratio compared with the control group.

Reduction in cell number could be due to reduced activity of the nuclei and thus reduced cell activity. Lai and colleagues (2004) showed that electromagnetic waves can induce harmful effects on the cell genome, reduce DNA repair capacity and increase necrosis and apoptosis particularly in the brain cells (27). The microwaves also affect radiation on the cell membrane and after influencing glycoproteins, they affect intracellular processes including the enzymes inside the cell cytoskeleton and the cell nucleus, they affect cell function and survival as well. The destruction of granular cells as the most cerebellar cortex cells can explain the reason for cerebellar weight loss (28). Since harmful stimuli such as heat and radiation can cause irreparable damage to the cell DNA and initiate pathways of cell suicide, the action mechanism of electromagnetic fields, cause local increase of temperature in the waves place due to their high energy or can cause damage as ionizing radiation due to the effects of free radicals. Free radicals also attack lipids and change their nature and may cause damage to the cell membranes by breaking protein binding. These radicals with peroxidation lipid increase short-chain fatty acid derivatives and malondialdehyde as a byproduct. It can also lead to oxidation of amino acids, creation of protein - protein binding, and breaking the DNA hydrogen bonds. So, maybe the changes in number of nerve cells in the cerebellum are caused by changes in DNA structure (1, 6, 10). On the other hand, some other studies have shown that electromagnetic radiation alters biophysical properties of membranes of brain cells including increasing the permeability rate of carbonic anhydrase (29), stimulation of Ca2 +-dependent K + channel activity by increasing the concentration of Ca2 + (30) and activation of sodium channels of the cerebellar granular cells by increasing theconcentration of arachidonic acid (31), Hence, it can be inferred that voltage changes can enhance cerebellar cells especially Granular to necrosis and apoptosis.

In our study, these changes were in the form of reduction in the number of cells as well as cerebellum weight loss. The results also revealed that cell phone radiation for 8 hours has a significant effect on weight gain in rats and could be due to stimulation in the secretion of growth hormone receptors in the hypothalamus and the pituitary gland or decreased secretion of hypothalamic thyrotropin-releasing hormone. This in turn may lead to decreased secretion of thyroid hormones and thus reduce metabolism and body weight gain (32). In general, the progress of science today ranging from electronics, computers, telecommunications and information technology, use of electromagnetic waves in different ways is growing astronomically; a prominent example is the rise of mobile devices. Unlike ionizing radiation, such as X-rays and gamma which their biological effects have been proven conclusively. Effects of non-ionizing radiation, like waves in the cell phone are still in the testing phase, so using these devices raises a fundamental question. Whether electromagnetic waves of these devices which have a high energy

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have an influence on the biological, and developmental processes, and ultimately do they affect human health or not? This questions leads to too many contradictory answers. Like many other studies, our study confirmed the biologically damaging effects. However, since the intensity, duration and usage of these devices vary depending on the location and culture of different communities. For example, more women than men use cell phone (33). It is therefore suggested that the effects of the waves be studied on other organs and on neurophysiology and neurobiology of cerebellar granular cells.

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