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Investigation of genetic stress parameters in brain tissues of rats exposed to 1.8 GHz cell phone radiofrequency electromagnetic field

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Abstract

Heat Shock Proteins (HSPs) may induce various cellular processes, including replication, apoptosis, cell-cycle progression. Mitogen-activated protein kinase (MAPK) cascades are the primary mechanism that mediates the cellular stress response to extracellular stimuli and regulates transcriptional activity. It has been shown that mobile phone exposure can stimulate the Hsp27/p38MAPK stress pathway. In this study, twenty-seven mature female Wistar albino rats were exposed to 1.8 GHz radiofrequency electromagnetic field (RF-EMF) 2h/day for 8 weeks (SAR: 0.06 W/kg). Hsp27 and p38MAPK gene expressions were investigated in rat brains. Rats were divided into groups sham-exposed, cage control, and 1.8 GHz RF-EMF exposed. Hsp27 and p38MAPK gene expression levels were investigated from the brain. p38MAPK expression was found to be upregulated in RF-EMF exposed group (p=0.018) Hsp27 expressions were not altered (p=0.897). In conclusion, long-term exposure to 1.8 GHz cell phone radiation can activate the Hsp27/p38MAPK stress pathway. It may cause several cellular disorders and can affect brain function.

Keywords: Electromagnetic radiation, public health, Hsp27, heat-shock proteins, p38 mitogen-activated protein kinases

Introduction

The earth's natural sources of electromagnetic radiation are associated with the earth's magnetic field, cosmic radiation, and local atmospheric electricity. Increasing radiofrequency (RF) electromagnetic radiation sources with the development of technology affect people in all areas of life [1]. The most common of these technological sources are mobile phones, Wi-Fi connections and base stations. Radiofrequency electromagnetic fields emitted from mobile phones vary between 800-2000 MHz [2]. Dual-band phones which cover the GSM networks at 1.8 GHz frequency are commonly in use in Europe, Asia, Australia, Brazil, and also in Türkiye [3].

It is known that cell phone radiation may adversely affect human health [4-7]. Heat shock proteins (HSPs) are induced by various stressors, such as oxidative stress, some transition of metals, and UV radiation. HSPs may play a critical role in the protection of cells against severe damage and cell death [8]. There is a lot of evidence suggesting that induction of HSPs by brief pretreatment of cells at elevated temperatures confers protection against the subsequent release of strong oxidants and other types of stress. HSPs are classified according to their molecular weight. These are small HSPs, Hsp90, Hsp70, Hsp100, and Hsp60 [9]. Hsp27 is generally synthesized against oxidative stress and produced by the HSPA1A gene which has been shown to trigger cancer [10,11]. Also, it has been reported cell phone radiation could trigger the expression of HSPs as a result of a cellular stress response [12].

MAPK (mitogen-activated protein kinase) cascades are the main response to multiple types of intrinsic and extrinsic

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cellular stresses. In response to stress, these cascades regulate transcriptional activity and control key processes of cell homeostasis such as death, proliferation, differentiation, and self-renewal [13]. It has been shown that prolonged exposure to mobile phone radiation can activate the Hsp27/p38MAPK stress pathway and cause various disorders in brain tissues [14,15]. Leszczynski et al. reported that 1-hour exposure of 900 MHz mobile phone radiofrequency radiation activate p38 MAPK and induced Hsp27 phosphorylation in human endothelial cell line. It causes an increase in blood-brain barrier permeability through the stabilization of endothelial cell stress fibers, leading to an increased risk of brain cancer development [14]. Kesari et al determined that 3G mobile phone radiation induces Hsp27, Hsp70 and p38MAPK phosphorylation by increasing oxidative stress in brain [15].

It is summarized in above that cell phone radiation causes oxidative stress in tissues especially in the brain [5-7]. MAPKs are known to be one of the main signal transduction pathways activated by oxidative stress. P38MAPK responds to various stresses and is activated by cell phone radiation. Also, Hsp27 has an important role in the intracellular defense system which is regulated by cellular stress conditions [8,10-12]. This information motivated us to determine the Hsp27 and p38MAPK gene expression levels of brain tissues in mature albino female rats upon exposure to 1.8 GHz cell phone radiation.

Material and Methods

Animals

Twenty-seven mature female Wistar albino rats were used in this study (Age: 10–12 weeks and average weight: 215±18 g) [16]. The rats were obtained from the Animal Research Laboratory, Mersin University, Mersin, Türkiye. Animal RF-EMF experimental procedures were approved by the Animal Experiments Ethics Committee of Mersin University (2014-HAYDEK-01). Animals were housed under standard laboratory conditions (Temperature: 23±1°C, humidity: 50±5%, with a 12h/12h light/dark cycle) and were given an ad libitum diet. They were divided randomly into three groups (n=9) cage control, sham-exposed, and RF-EMF exposed. Before starting the RF-EMF exposure, the rats in the sham-exposed and RF-EMF-exposed groups were put into the pie cage restrainer 2 hours per day (Between 08.00 am to 10:00 am local time in Türkiye) for one week to adapt them to experimental conditions. The cage control group was kept under standard animal laboratory conditions. Sham exposed group was kept under the same conditions as the RF-EMF-exposed animals without RF-EMF exposure, for assessing the stress of staying in the restrainer. Also, RF-EMF exposed group was exposed to 1.8 GHz RF-EMF, 2 hours per day for 8 weeks (Between 08.00 am to 10:00 am local time in Türkiye). At the end of the eighth week, the animals were sacrificed under anaesthesia (ketamine 90% mg/kg, ksilazin 10% mg/kg) brain tissues were removed immediately on an ice block and stored at -80°C until tissue homogenization.

Electromagnetic exposure

In this research, RF-EMF application was carried out using a 1.8 GHz emitter (1800 CW2; Everest, Adapazarı, Türkiye). The device works at 217 Hz modulation. Emits intermittent or continuous waves. It has 2 RF output channels and 1.8 GHz, with a maximum output power of 2W. There is 1 monopole antenna. 1.8 GHz RF emitter was set to 0.2 W output power and a continuous wave mode during RF exposure. RF-EMF exposure conditions were determined by Biophysics researchers. The Electrical and Electronics Department recorded the electric field measurements of the RF-EMF system at the beginning and end of the study. An Electrical Field Meter (PMM 8053, Elettroniche Centro Misure Radioelettriche, Savano, Italy) was used for measurements. Rats were placed in a 5-slice cake cage holder (42 x 20 x 7 cm) and RF-EMF exposure was applied in the middle in a vertical position. Stress on the treatment group mice was reduced by creating a series of air holes about 2 cm in diameter on the top surface of the cake cage holder. Figures 1A and 1B show the rat exposure design. The experimental group was placed in the restrainer and exposed to RF-EMF at 1.8 GHz. This application was carried out at the same time every day at 5–9 V/m (6.8±0.1) 2 hours/day for 8 weeks. The specific absorption rate (SAR) was used for the dosimetry. The electric field measurements were taken from the whole body (the head, dorsal, and tail regions) of each rat for 1 minute during the first and the last week of the study. The SAR was calculated according to a previous study [17].



Figure 1A. 1.8 GHz emitter (1800 CW2; Everest, Adapazarı, Türkiye) **1B.** RF-EMF exposure application of rats is made in the center of the pie cage restrainer consisting of 5 slices in vertical position

Gene expression analysis

Total RNA isolation

Homogeneous brain tissues were quickly prepared on ice block with a sterile scalpel. 50 mg tissue homogenate was taken from total homogeneous tissues and also homogenized with MagNA-Lyser Green Beads (Roche Life Science, Germany). Total RNA was extracted from brain tissue homogenates with Purelink RNA Mini Kit (Ambion, Thermo Fisher Scientific, Germany).

Reverse transcription

Complementary DNA (cDNA) was synthesized from total RNA by using the cDNA Reverse Transcription Kit (Ambion, Thermo Fisher Scientific) according to the manufacturer's instructions.

Real-time PCR

Analysis was performed with a High-Capacity Real-Time

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PCR System (ViiA™ 7 Applied Biosystems, Germany). After determining cycle threshold (CT values via gene specific probes (TaqMan Gene Expression Assay, Germany), ΔΔCT values were calculated with ViA7tm software. TaqMan Gene Expression Master Mix and TaqMan Gene Expression Assays were used to prepare the reaction mix and actin beta was used as an endogenous control for gene expression analyses. Hsp27 (Rn00687529), and p38MAPK (Rn00578842) expression levels were determined with TaqMan Gene Expression Assays. All reactions were taken in triplicate.

Statistical analysis

Shapiro-Wilk test was used to determine whether all parameters were normally distributed or not. Kurskal-Wallis test was used to test the differences between the groups for each parameter. Bonferroni-Dunn test was used for posthoc comparisons. Mean±standard deviation or Median [minimum-maximum] was calculated in each group for all parameters according to the normality assumption provided. Benjamini-Hochberg critical value was accepted as 0.05 for the comparisons and P values were adjusted. The results for $p<0.05$ were accepted as statistically significant.

Results

SAR calculation

The SAR value was calculated using the measurements in the formula below. The calculated SAR was 0.06 W/kg (Table 1).

$$SAR = \sigma \cdot \frac{E_{RMS}^2}{\rho} \qquad (W/kg)$$

E_{RMS}: Electric field (the root mean square electric field), (V/m)
σ: Mean electrical conductivity (S/m)
ρ: Tissue mass density (kg/m^3)

Gene expression analyses

CT values determined via gene-specific probes than ΔΔCT values were calculated with ViA7^{tm} software. Analyses showed that p38MAPK gene expression was increased in the RF-EMF-exposed group compared to the sham-exposed (adj p*=0.018). There was no difference between cage-control and RF-EMF-exposed rats. Also, no difference was found between the sham-exposed and cage-control groups. There were no differential expression levels of Hsp27 (adj p*=0.897) between the groups. Median [minimum-maximum] values of gene expression levels were shown in Table 2.

Table 1. Calculation of electric field and SAR

	Tissue bulk density (kg/m^3)	Average electrical conductivity (S/m)	Measured Average Electric Field Value (V/m)	SAR (W/kg)
Whole body	1040	1.389380	6.8±0.1	0.06

* Electrical conductivity values for 1.8GHz frequency were obtained from http://www.fcc.gov/oet/rfsafety/dielectric.html.

Table 2. ΔΔCT gene expression levels measured in brain from experimental groups and p-values from Kruskal-Wallis Test

	1.8 GHz RF-EMR Exposed group	Sham group	Control group	Adj. P*
	Median (Min-Max)	Median (Min-Max)	Median (Min-Max)	
Hsp27	2.332 (0.619-30.675)	2.058 (0.283-6.732)	2.472 (0.946-8.988)	0.897
p38MAPK	0.772 (0.445-2.922)	0.383 (0.243-1.476)	0.524 (0.253-1.964)	0.018

*Benjamini–Hochberg adjusted p values

Discussion

Recent studies show that RF-EMF exposure affects a wide range of biochemical and physiological processes in several organs. It is particularly known that long-term RF-EMF exposure can cause oxidative stress and also can alter some heat shock gene expressions in different types of cells. Ibitayo et al. (2017) reported that weak fields can accelerate electron-sharing processes and consequently weaken H-bonding in cellular macromolecules [18]. This finding may explain the molecular and biochemical changes in the cells which are exposed to RF-EMF in the long term. Based on recent research we aimed to determine the effects of cell phone usage on the brain tissues of rats.

The effects of cell phone radiation exposure on brain tissues are explained in different molecular studies which are focused on histopathological, epigenetic, and biochemical changes. One of them showed that after two months 900 MHz and 1800 MHz mobile phone exposure causes histopathological changes in adult male rat's brains. These changes were in the nerve cells especially also in the cerebral and cerebellar cortices. These changes were more pronounced in those exposed to 1800 MHz compared to the others [19]. Singh et al. determined the proteome profile in rat hippocampus exposed to 3G (1964.7 MHz) mobile phone radiation for 20 weeks. They identified important proteins involved in either energy metabolism, and transmembrane ion pumps or are markers of neurodegeneration or protection in exposed groups [20]. Kumar et al. focused on epigenetic changes in the hippocampus and reported that 900 MHz, 1800 MHz, and 2450 MHz cell phone radiation (2h/day, one, three, and six-month exposure) causes alteration in epigenetic modulation which alters gene expression, 2450 MHz exposure for six months was more critical. They determined alteration in DNA methylation was decreased and histone methylation was increased in the rat hippocampus [21]. Tohidi et al. also

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investigated affected hippocampus tissues that were exposed to 900MHz and 1800MHz electromagnetic radiation 0.5, 1, 2, and 4 hours twice a day for 30 days. They determined that cell phone exposure can cause considerable changes in the balance of Bax/Bcl2 mRNA expression in the hippocampus of mice [22]. Also, there are many in-vivo and in-vitro studies on brain tissue have demonstrated molecular alterations that are stimulated with cell phone radiation [5-7,23].

In this paper, we determined the expression levels of Hsp27 and p38MAPK genes in all brain tissues of rats exposed to 1800MHz RF-EMF. P38MAPK plays critical roles in response to various stresses and is activated by cell phone radiation. MAPKs are known to be one of the main signal transduction pathways activated by oxidative stress. Also known that cell phone radiation causes oxidative stress in tissues especially the brain [5-7]. MAPK signaling regulates gene expressions that are active in several cellular processes survival, proliferation, and cell death. We determined the expression level of the p38MAPK gene in the whole brain tissue of rats exposed to the experimental protocol and found that there was a significant increase in those exposed to 1.8 GHz RF-EMF compared to the other groups. We determined that 1.8 GHz RF-EMF exposure caused cellular stress in brain tissues and stimulated the p38MAPK pathway. The well-known heat shock protein Hsp27 is a target gene that has an important role in the intracellular defense system regulated by cellular stress conditions and heat shock. Hsp27 protects cells from cell death by inhibition of caspase-dependent apoptosis, so that it has an anti-apoptotic agent under stress conditions by interacting mitochondrial dependent and independent pathways [24]. In-vivo and in-vitro studies have shown that cell phone radiation causes changes in many genes expression levels and protein synthesis including heat shock proteins which are involved in cell proliferation, apoptosis, and survival [25-28]. In our study, we determined that the expression level of this gene did not change between groups. When the Hsp27/p38MAPK pathway is stimulated, an increase in the expression of both genes is expected. However, in this study, while p38MAPK expression increased, no significant increase was observed in Hsp27 expression. It was determined that the expression levels of these two genes increased in human endothelial cells [14] that were exposed to mobile phone radiation and in the brain tissues of rats [15]. These studies reported that mobile phone exposure induced oxidative stress which activated the Hsp 27 and activated p38MAPK cell death signaling [14,15]. In this paper, we determined an increases on gene expression level of p38MAPK but not Hsp27. We agreed that the earliest stress response to RF-EMF appears to come from p38MAPK expression. A methodological limitation of this study is that p38MAPK and Hsp27 protein levels were not measured and detected in brain tissues. Although p38MAPK mRNA level is increased, not all of the transcript may be converted into protein. Also epigenetic factors may affect the expression of the Hsp27 gene should be revealed by further studies.

Conclusion

It was concluded that 1.8 GHz RF-EMF for 8 weeks (2h/day) altered the p38MAPK gene expression level but not the Hsp27 gene expression. It seems that 1.8 GHz RF-EMF is recognized as a stress factor by brain cells. Epigenetic factors may have contributed to the lack of increased expression of the Hsp27 gene. In conclusion, chronic exposure to 1.8 GHz RF-EMF may lead to p38MAPK activation and may cause damage and dysfunctions in brain tissue.

Conflict of Interests

The authors declare that there is no conflict of interest in the study.

Financial Disclosure

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Ethical Approval

Animal RF-EMF experimental procedures were approved by the Animal Experiments Ethics Committee of the Mersin University (2014-HAYDEK-01).

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