EXPERIMENTAL STUDY

The effect of exposure to 1800 MHz radiofrequency radiation on epidermal growth factor, caspase-3, Hsp27 and p38MAPK gene expressions in the rat eye

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ABSTRACT

OBJECTIVE: Radiofrequency electromagnetic fields (RF-EMF) may induce DNA damage and oxidative stress in human lens epithelial cells (LECs). We aimed to investigate the expression levels of heat shock protein 27 (Hsp27), p38 mitogen-activated protein kinase (p38MAPK), epidermal growth factor receptor (EGFR) and caspase-3 gene expression levels in rat eye that was exposed to 1800 MHz RF-EMF.

METHODS: Thirty-seven female Wistar albino rats were divided into three groups. The rats in the study group (n = 9) were exposed to 1800 MHz RF-EMF at an electric field 6.8 ± 0.1 V/m and 0.06 W/kg specific absorption rate (SAR) for 2 hours per day for eight weeks. Sham group (n = 9) was kept under similar conditions as the exposed group without exposure to RF-EMF. The rats in all three groups were sacrificed and their eyes were removed. Hsp27, p38MAPK, EGFR, caspase-3 gene expression levels were investigated in detail with real-time polymerase chain reactions (Real-Time PCR).

RESULTS: caspase-3 and p38MAPK gene expression were significantly upregulated in the ocular tissues following exposure to RF-EMF (p < 0.05).

CONCLUSION: According to our findings, eye cells recognize EMF as a stress factor, and in response, activate caspase-3 and p38MAPK gene expressions. These results confirm that RF-EMF can cause cellular damage in rat ocular cells (Tab. 2, Fig. 3, Ref. 37).

KEY WORDS: radiofrequency radiation, rat eye, gene expression, caspase-3, p38MAPK.

Introduction

Increasing use of communication devices such as mobile phones, laptops, data transmission and wireless… etc. causes chronic electromagnetic field (EMF) exposure in daily life. Especially, because of increasing use of mobile phones associated with developing technology, people are often exposed to a range of ecological pollution. 1800 MHz radiofrequency radiation (RF-EMF) used for mobile phone communication, especially during talking, may emit at a low level electric field absorbed by user’s body particularly head and the eyes (1). Evidence showed that RF-EMF changes gene expression levels, cell cycle control, apoptosis and tumourigenesis (2, 3, 4).

When RF-EMF waves encounter with biological systems, the magnitude of electromagnetic field effects on biological system is dependent on some physical parameters of the electric field, specific absorbed rate dose, dielectric properties, for example tissue conductivity, of biological system. These parameters may cause thermal effect depending on thermal stress at very high peak specific absorption rates (SAR) (tens of W/kg) and non-thermal when electric field is below 10V/m and/or SAR is below from 0.1 W/kg (1, 5, 6). Thus, the exposure studies and the reports of RF-EMF on biological system at low level electric field, low level SAR and non-thermal effects are ongoing and are becoming very significant, especially on non-thermal effects.

The exposure of the ocular system is very important as the mobile phone is close to the eyes during mobile phone communication. Also it has the potential to damage the eye tissue especially the lens and is considered a risk factor for cataracts and also uveal melanoma (7, 8, 9, 10, 11).

It is known that EMF enhances the expression levels and phosphorylation of Hsp27 that is a part of the small Hsp family, and it has a defensive property in reaction to stress and it is believed that Hsp27 is involved in cancer development (8, 12, 13, 14, 15). Within the intracellular responses mediated by EMF, the MAPK signaling cascade family is the crucial candidate and it has been shown that long exposure to RF-EMF can activate p38MAPK...
(12, 14, 15). Epidermal growth factor (EGF) is a multifunctional cytokine that affects the mammalian visual surface (16, 17). The epidermal growth factor receptor (EGFR) is expressed in the ocular surface epithelium, especially in basal epithelial cells in the cornea, limbus and conjunctiva (18, 19). Caspase-3 is a key member of the caspase family of proteases. Caspase-3 exists in cells as an inactive proenzyme (20, 21). It has been known that RF-EMF may affect biological systems by increasing free oxygen radicals, apoptosis, and mitochondrial depolarization levels (22, 23).

To our knowledge, there is no report about EGF, caspase-3, Hsp27 and p38MAPK gene expressions in the eye tissue. Herein, we investigated whether exposure of 1800 MHz RF-EMF could alter the Hsp27, p38MAPK, EGFR and caspase-3 gene expression levels in the rat ocular tissue.

Materials and methods

Animal experiments

27 Wistar Albino female rats aged between 8–12 weeks with initial average weight of 215 ± 18 g were kept in Animal Research Laboratory, Mersin University. They were fed ad libitum and housed in acrylic cages in an animal room with a 12 h light/12 h dark cycle. Temperature and relative humidity were maintained at 23 ± 1 °C and 50 ± 5 %, respectively. All of the animal measures were confirmed by the Mersin University Animal Experiments Ethics Committee (2014-HAYDEK-01).

The Nelder–Mead method was used for at least 7 in the Wistar albino rats, and a total of 21 rats were planned to be included in each group of female rats in the study (24). Considering the 20 % loss, 27 rats total were included in the study (9 rats per group). We divided the rats into three groups: control, sham and exposed. The control group was kept in normal conditions, sham group was kept under the similar conditions with exposed group without exposure to RF-EMF and the study group was exposed to 1800 MHz RF-EMF radiation at electric field 5–9 (mean: 6.8 ± 0.1)V/m, for 2 hours per day for eight weeks.

Electromagnetic exposure

The electromagnetic radiation exposure system was designed by the Department of Biophysics according to previous studies (25, 26). The restrainer and RF-EMF measurement device used to expose the rats to the RF-EMF are illustrated in Figure 1.

The exposed group was placed inside the restrainer, and the rats were exposed to the EMF 5 – 9 V/m (mean: 6.8 ± 0.1) for 2 h/day for 1 W at the same time every day. SAR was used for the dosimeter. The electric field measurements, which are used for SAR calculation, were taken from the head, body and tail of each rat during exposure for 1 min. The SAR was calculated according to previous studies (26, 27). The calculated SAR was 0.06 W/kg (Tab. 1).

Calculation of electric field and SAR

\[
SAR = \frac{E_{RFEM}^2}{\rho} (W / kg)
\]

\[E_{\text{RFEM}}\]: Electric Field (the root mean square electric field), (V/m) 
\[\rho\]: Tissue bulk density (kg/m³)

Gene expression analysis

Total RNA Isolation

The rats were sacrificed under anesthesia at the end of the exposure periods. Their eyes were removed and stored at −80 °C until RNA extraction. Total RNA was extracted from rat ocular tissue with Purelink RNA Mini Kit (Ambion, Thermo Fisher Scientific).

cDNA Synthesis

cDNA was synthesized from total RNA with High-Capacity cDNA Reverse Transcription Kits (Ambion, Thermo Fisher Scientific). 20 μl reaction mix included 2.0 μL 10X RT Buffer, 0.8 μL 25X dNTP Mix (100 mM), 2.0 μL 10X RT Random Primers, 1.0 μL MultiScribe™ Reverse Transcriptase, 10.2 μL Nuclease-free H₂O, 4μL cDNA. The reaction incubated at 25 °C/10min, 37 °C 120 minutes, at 85 °C 5 minutes, and final hold was at 4 °C. The reaction was performed in a thermal cycler from Techne Prime.

Real-Time PCR

Real-time PCR was performed in a High-Capacity Real-time

| Tissue density (kg/m³) | 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 1800MHz frequency were obtained from http://www.fcc.gov/oet/rfsafety/dielectric.html
PCR System (ViA 7™). 20 μl PCR mix included 10.0 μL TaqMan Gene Expression Master Mix (2), 1.0 μL TaqMan Gene Expression Assay (20), 2.0 cDNA template, 7.0 Nuclease-free H2O. Thermal cycle conditions for uracil DNA glycosylase (UDG) incubation at 50 °C for 2 min, AmpliTaq Gold, UP Enzyme Activation at 95 °C for 10 min, followed by 40 cycle denature 95 °C for 15 sec and Anneal/Extend for 60 °C for 1 min. Reference sample was made by mixing the control group RNAs. Reactions were incubated in a 96-well plate. Actb (actin beta) (Rn00667869) were used by endogenous control in Real-Time PCR. Hsp27 (Rn00687529), p38MAPK (Rn00578842), EGFR (Rn00563336) and the caspase-3 (Rn00563902) genes expression levels determined with TaqMan Gene Expression Assays. All reactions were taken in triplicate. Analyses were performed with ViIA™ 7 Software (Applied Biosystems).

**Statistical analysis**

The percentage for categorical data frequency and the statistical evaluation of data obtained from the study, the mean ± standard deviation or median depending on the distribution pattern, was continuous data (min–max) summarized terms. Parametric numeric variables (one-way analysis of variance (ANOVA) testing) and non-parametric methods (Kruskal–Wallis test) were used. Differences were considered significant at p < 0.05 and p < 0.01, respectively. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows v.22.0).

**Results**

After determining CT values via gene specific probes (TaqMan Gene Expression Assay) ∆∆CT values were calculated with ViA7™ software.

**Tab. 2. Comparison of the mean values of the studied gene expressions in control, sham and experimental groups.**

|               | Exposed group | Sham group | Control group | Adj. p*  |
|---------------|---------------|------------|---------------|----------|
|               | Mean=SD       | Median (Min–Max) | Mean=SD       | Median (Min–Max) | Mean=SD       | Median (Min–Max) |          |
| Caspase3      | 6.868±11.681  | 1.589 (0.396–35.163) | 0.254±0.221   | 0.210 (0.029–0.759) | 1.785±3.998   | 0.370 (0.091–10.845) | 0.008 |
| Egfr          | 2.234±4.063   | 0.412 (0.151–0.726) | 0.407±0.200   | 0.210 (0.029–0.759) | 0.512±0.114   | 0.230 (0.078–0.669) | 0.130 |
| Hsp27         | 0.775±1.328   | 4.118±12.155   | 0.155±0.200   | 0.102 (0.001–0.689) | 0.061±0.052   | 0.052 (0.012–0.139) | 0.162 |
| p38MAPK       | 0.474±0.676   | 0.141±0.212    | 0.479±0.676   | 0.141±0.212    | 0.029±0.046   | 0.015 (0.003–0.134) | 0.030 |

*Benjamini–Hochberg adjusted p values
software (Fig. 2). Our results showed that the long-term exposure of 1800 MHz RF radiation may affect some genes expression levels such as caspase 3 (Adjp* = 0.008), p38MAPK (Adjp* = 0.030) in the rat ocular tissue. Post hoc test was used to determine that caspase 3 and p38MAPK genes expression levels were significantly altered in 1800 MHz RF-EMF exposed group compared to sham group and control group (Adjp* < 0.005).

However, EGFR (Adjp* = 0.13) and Hsp27 (Adjp* = 0.162) genes expression levels were not altered by long term exposure to 1800 MHz RF radiation in the exposed group compared to sham group and control group. Comparison of four genes expression levels in all groups is detailed in Table 2. It is also shown graphically in Figure 3.

**Discussion**

To date, a number of investigations have been performed to assess human health risks especially the genetic and cytotoxic effects induced by radiofrequency radiation emitted from mobile phones. High-frequency EMF from mobile phones and other modern devices has the potential to damage eye tissues; however, its impact on the gene expression levels of some proteins is undefined at present. Investigations showed that RF fields may cause alterations in the conformation of cellular proteins and the eventual synthesis of stress response proteins (13, 28, 29). In this study, we investigated whether 1800 MHz RF-EMF alters Hsp27, p38MAPK, EGFR and caspase-3 gene expression levels or not in the rat eye tissue.

There are several published studies showing that electromagnetic fields can damage human DNA, have an effect on protein phosphorylation and activity of stress proteins and kinases and also can alter expression levels of apoptotic genes in various tissues (13, 30, 31, 32). Actually, biological effects of cell phone radiofrequency radiation are still uncertain. Liu et al described that exposure to EMF of 1950 MHz might not promote tumor configuration, but continuous exposure damaged the mitochondria of astrocytes and induced apoptosis through a caspase-3-dependent pathway with the involvement of bax and bcl-2 (31). Subsequently, Zhijian et al published microarray data, 17 proteins (e.g. p73, BIM, Bak, Caspase 1, EGFR, IGF-1R, EGFR8, CD30) with differential expression were found in lymphoblastoid cells after 1.8 GHz FR-EMF exposure. Those proteins are mostly in the process of DNA damage repair, apoptosis, oncogenesis, cell cycle and proliferation (30). Calabro et al studied the biological damage caused by GSM mobile phone frequencies by assessing EMF while using a mobile phone (33). They evaluated cell stress response via MTT assay and used changes in Hsp expression (Hsp20, Hsp27 and Hsp70) and caspase-3 activity levels as biomarkers of apoptotic pathways. The researchers established that neither cell viability, Hsp27 expression nor caspase-3 activity were significantly changed, but a significant decrease in Hsp20 expression was observed at both times of exposure, whereas Hsp70 levels were significantly increased only after 4 h exposure. They concluded that the modulation of the expression of Hsps in neuronal cells could be an early response to 1750 MHz EMF radiation and 0.08 W/kg SAR value.

Stang A. found a relation between cell phone radiation and eye cancer (7). The study investigated a type of eye cancer called uveal melanoma, in which tumors form in the layer that makes up the iris and the base of the retina. The research suggests there is a threefold increase in eye cancer in people who regularly use these devices. Bormusov et al found a relation between microwave radiation and the growth of cataracts. This study reports that extended contact with microwave radiation related to that used by mobile phones can cause both macroscopic and microscopic injury to the lens, and that at least part of this injury seems to accumulate over time and does not seem to repair itself (9). Even though numerous incompatible views have been expressed concerning changes in Hsp expression induced by EMF, inadequate results have been reported in mammalian eye cells (34, 35). Yao et al determined that DNA damage induced by 1.8 GHz radio frequency radiation for 2 h in human lens epithelial cells (hLECs) occurred (36). Lixia et al found that contact with non-thermal dosages of RF from wireless devices at 1.8 GHz can induce no or repairable DNA damage; further, increased Hsp70 protein expression in hLECs occurred without change in the cell proliferation rate (37).

Previous studies have established that RF-EMF emitted from mobile phones could be connected with alterations in cancer via a chronic activation of the heat shock response (2, 8, 13, 33, 35). According to the present study, it seems that sham eye cells recognize EMF as a stress factor, and in response, activate p38MAPK and caspase-3 gene expression to destroy unwanted damaged cells. These results confirm that high-frequency RF-EMF can damage human eye cells. In conclusion, we suggest that RF-EMF can be an external stress factor that causes cell damage in eye tissue.

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