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The Effects of Radiofrequency Electromagnetic Field on Brain-Derived Neurotrophic Factor Protein Expression of Human Astrocytes

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Abstract

Mobile phone and wireless gadgets have become an essential component of daily life. Potentially each individual is exposed to electromagnetic field (EMF) radiation produced by mobile phone or wireless gadgets for at least a few hours per day. The EMFs produced are known as radiofrequency EMF (RF-EMF). The biological effects of RF-EMFs have been demonstrated in numerous in vivo and in vitro studies. In human tissues, a number of researchers have demonstrated the RF-EMFs affect the proliferation of cells and lead to programmed cell death (apoptosis). Brain-derived neurotrophic factor (BDNF) is growth and differentiation factor which involve in the development of new neurons and sypnases. It roles in the pathophysiology of brain disease has been implicated.

***Aim** : In here, we aimed to investigate the effects of 900 MHz and 1.8 GHz of RF-EMFs on the expression of BDNF in cultured normal human astrocytes.*

***Methodology** : We exposed the normal human astrocytes to 900 MHz and 1.8 GHz RF-EMFs for 2 hr, 6 hr, 18 hr, 48 hr and 72 hr. Then, cell proliferation assay (MTS assay) and DNA fragmentation assay*

were performed. In addition, BDNF protein expression was measured using ELISA.

***Results & Interpretation**: Our result showed that RF-EMF at frequency 900 MHz and 1.8 GHz affect the proliferation rate of cells but no enough to cause apoptosis. While, olny 48 hr of 1.8 GHz exposure could affect the normal level of BDNF protein expression in normal human astrocytes.*

Keywords : Brain neurotrophic factor, Electromagnetic field, Human astrocytes, Radiofrequency

Introduction

Nowadays, almost every individual would own mobile phone and other wireless gadgets. These devices are held close to their body and being used regularly. Therefore, an individual potentially faces daily exposure to electromagnetic field (EMF) radiation produced by mobile phone or wireless gadgets. Both devices usually transmit and received electromagnetic wave between 800 to 1900 MhZ [1]. EMFs produce by both devices are better known as radiofrequency EMF (RF-EMF). Due to the exposure, there are growing concerns for any potential effects which could be detrimental to the human health.

In the light of research, the biological effects of RF-EMFs have been reported in human tissues. Devices that produced RF-EMFs are usually positioned close to organ such as nervous and reproductive system [2]. Therefore most of the reported RF-EMF's biological effects are focusing on tissue or cells related to brain and reproductive organs. Although numerous studies had demonstrated the biological hazards of RF-EMFs, yet further understanding of RF-EMFs effects on other organs and involvement in the pathogenesis of certain diseases are sought to be investigated. Moreover, there were diversities and conflict in results reported from in vivo and in vitro studies for RF- EMFs.

In brain, Zhao *et al*. (2007) demonstrated that EMFs activate different genes in primary culture of neuron and astrocytes [3]. While others shown the involvement of EMF in altering brain cognitive functions [4] and expression of genes for different part brain tissues [4,5]. Eberhardt *et al*. (2008) showed significant neuronal cell damage after being exposed to radiation from a global system mobile communications (GSM) phone at the frequency of 915 MHz for 2 hr/day for 14 days and 28 days [6]. This study was supported by the findings of other investigators [4]. Eyes and nose are known as doors to the brain. Thus, a number of studies have reported the effects of RF-EMFs on cells from those two facial structures. The eye is the easy target for RF-EMF.

Furthermore, damage of the eye could lead to damage of general visual-CNS function. However no significance change was detected in retinal ganglion cells exposed to RF-EMFs at frequency 900 and 1800 MHz [7]. Similarly, no change was observed in the nasal area of animals [8] and human [9] exposed to RF-EMFs.

In reproductive system, pregnant rats exposed to the frequency 915 MHz did not show significant changes in litter size, fetal weight and maternal weight [10]. 101 replicated the study on chicken embryos and reported an increase in cell death of RF-EMFs exposed compared to untreated cell [11]. In male rats exposed to 915 MHz frequency for 2hr/day for 30days did not exhibit any significant alteration in sperm counts and motility [12,13]. In contrast, epidemiological study reported that exposure to GSM phone for 6hr/day for 5 days lead to decrease sperm quality. In another study, mice exposed to same frequency for 1hr/day for 24 months showed no significant [14].

Studies of RF-EMFs on an in vitro model are mostly looking at the effects RF-EMFS on DNA damage and cell signaling. Lymphoblastic leukemia cells upregulated expression of pro-surviving genes and when exposed to short duration of 900 MHz [15]. Meanwhile, in another study, lymphoblastic leukemia cells induced expression of DNA repair and cell cycle arrest genes after long and short duration of 900 MHz exposure. In contrast, others failed to replicate the result in in vitro model of cancer cells or glial cells [4,16]. From a clinical standpoint, roles of BDNF have been implicated in the pathophysiology of various brain diseases. The stress-induced steroid hormone, glucocorticoid, and BDNF are putatively associated with the pathophysiology of depression.

Brain-derived neurotrophic factor (BDNF) is growth and differentiation factor which involve in the development of new neurons and sypnases. It roles in the pathophysiology of brain disease has been implicated. Moreover, it has been associated with the development of depression. Here, we aimed to investigate the effects of RF-EMF on the BDNF level of normal human astrocytes.

Materials and Methods

Norman human astrocytes (NHA) (Lonza, USA) were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (Gibco- Life Technologies, USA), penicillin and streptomycin. They were grown in incubator at 37oC with 5 % CO2 until 80-90%. The cells were harvested with trypsin (Gibco- Life Technologies, USA) and counted. They

were then plated into six well plates and incubated overnight. Each plate was exposed to 2 frequencies of RF-EMF; 900 MHz and 1.8 GHz at timepoint of 2 hr, 6 hr, 18 hr, 48 hr and 72 hr.

MTS assay (Promega Co., USA) was performed to study cytotoxicity and cell viability. For this assay, cells (1.5 x 104 cells/well) were seeded in a 96 well plate prior to exposure. 20 µL of CellTiter 96® AQueous One Solution Cell Proliferation Assay were added and incubated in the dark for one and half hour in 5% CO2 incubator. The plates were read using Plate Reader (Perkin Elmer, USA) at 490 nm. Percentage of cells viability was calculated based on the following formula: (A-B) /A x 100%, where A was the absorbance value for untreated cells while B was the absorbance value for treated cells.

DNA fragmentation in all untreated and treated cells was determined using the DNA fragmentation ELISA kit from Roche Molecular Biochemical (Germany). Cells were cultured in six well plates at a density of 1 x 106 cells and incubated overnight at 37°C in 5% CO2. The medium was removed and replaced with new medium containing 10 µM BrdU labelling solution. The cells were incubated with the BrdU-containing medium overnight at 37°C in 5% CO2 and 95% humidity. The next day the medium was removed and cells were lysed with 200 µl 1 x incubation solution (Solution 5) and incubated for 30 minutes at 15-25 °C. 100 µl of the supernatant was transferred into a microtiter plate coated with anti-DNA antibody and incubated for 90 minutes at room temperature. The wells were washed three times with washing. After washing, the DNA were fixed and denatured by microwave irradiation for 5 minutes on medium power (500W). The, the microtiter plate was cooled for 10 minutes at -20°C. 100 µl of anti-BrdU-POD conjugated solution was added into each well of the microtiter plate. The microtiter plate was covered tightly and incubated in the dark for 90 minutes at 15 - 25°C. The wells were washed again with washing solution and incubated for 30 minutes at room temperature. The absorbance was measured at 370 nm wavelength (with 492 nm as a reference wavelength) using a spectrophotometer (SpectraMAX 190, Molecular Devices). The increase in DNA fragments released by apoptotic cells is proportional with an increase in OD percentage of apoptotic cells was calculated.

The expression of Brain-derived neurotrophic factor (BDNF) was measured using ELISA kit obtained from Cusabio Biotech Co, Ltd (China). For this part, all

reagents and standards were prepared according to the kit instruction. In 96 well of microplate, 100 µL of standards or samples were added to each well and incubated for 2 hours at 37°C. Then, the solution was removed from each well and 100 µL of biotin-antibody was added, Plate was further incubated for 1 hour at 37°C. Then, wells were washed and substrate solution was added to each well prior to 30 minutes incubation at 37°C in the dark. Finally, stop solution was added to each well of the plate and the absorbance was read at 450nm. Percentage of BDNF protein expression was calculated based on the absorbance reading.

Results and Discussion

MTS is a colorimetric method used to represent the number of viable cells which undergoes proliferation. In here, MTS assay was employed to determine the effects of radiofrequency EMF (RF-EMF) on normal human astrocytes cell proliferation rate. As depicted in Figure 1A, the results show that 900 MHz frequency significantly ($p < 0.05$) increased cell proliferation at 48 hr exposure and decreased cell proliferation at 72 hr exposure. Meanwhile, cells show significant increase in cell proliferation rate at 2 hr, 6 hr, 48 hr and 72 hr of exposure to 1.8 GHz (Figure 1B).

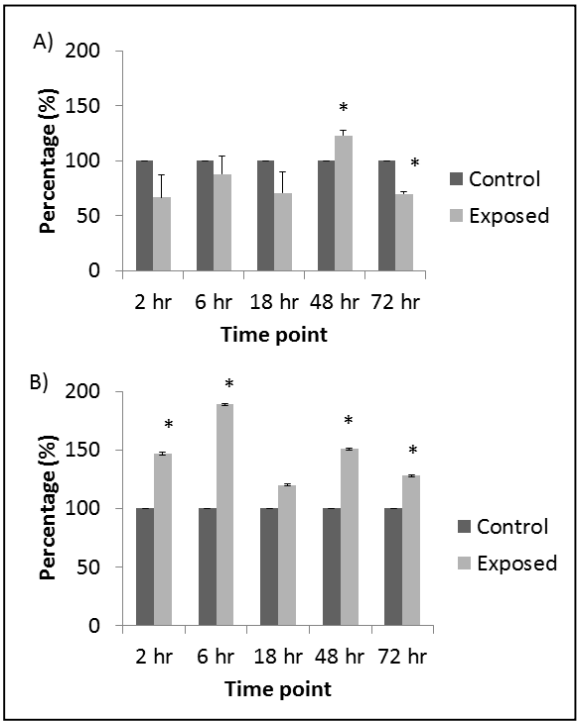

Figure 1 : Cell proliferation of human astrocytes exposed to 900 MHz (A) and 1.8 GHz (B) radiofrequency EMF from 2 hr to 72 hr. (*, indicated significant difference at $p < 0.05$ compared to control cells, n = 3 independent experiments).

In our study we only observed significant reduction in cell proliferation at 72 hr exposure with 900 MHz RF-EMF. This result is similar to the other 2 studies reported by Sarkar et al. (1994) [16] and Lie et al. (2012) [17]. In contrast, significant increase in cell proliferation was observed in 900 MHz RF-EMF cells exposed for 48 hr and 1.8 GHz RF-EMF cells exposed for 2hr to 72 hr. Similarly, Sul *et al.* (2006) observed increase in cell proliferation of C6 glial cells exposed

to RF-EMF from day 1 to day 14 of exposure [18]. Furthermore, they concluded that greater increase was observed in cell with lower initial seeding density compared to higher seeding density (more confluent).

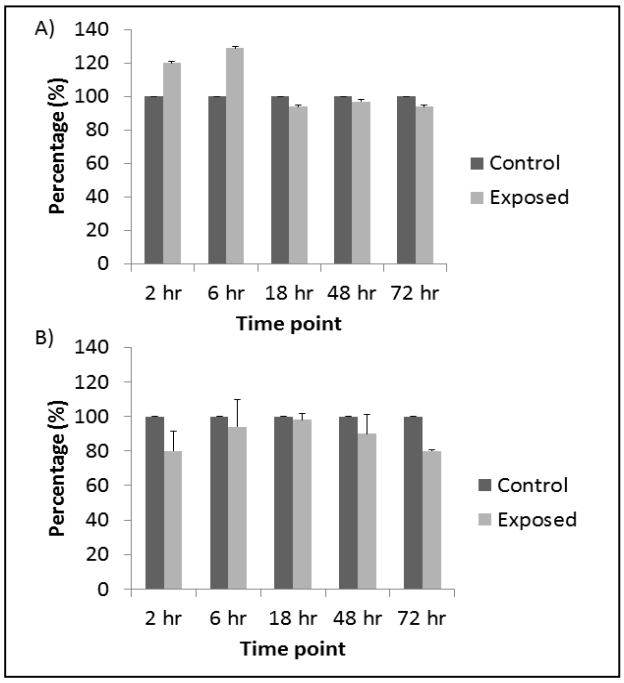

Figure 2: DNA fragmentation of human astrocytes exposed to 900 MHz (A) and 1.8 GHz (B) radiofrequency EMF from 2 hr to 72 hr. (*, indicated significant difference at $p < 0.05$ compared to control cells, n = 3 independent experiments).

The break-up of genomic DNA arises as result of cellular nucleases activity. This break-up of genomic DNA is a feature of cell death which occurs via a programmed cell death known as apoptosis. DNA fragmentation assay was employed to determine whether normal human astrocytes exposed to 900 MHz and 1.8 GHz activate apoptosis. In here, we did not observed any significance changes in DNA fragmentation between control and exposed cells. Similarly, Chen *et al*. (2014) did observed any differences in DNA fragmentation of unexposed embryonic neural stem cells to the other groups exposed to 1.8 GHz for 48 hr and 72 hr [19]. In contrast, Campisi *et al*. (2010) demonstrated an increase in DNA fragmentation of rat cortical astrocytes exposed to 900 MHz for 20 min/ day for 14 days [20]. These discrepancies in result for DNA fragmentation could be due to the duration of cells being grown on a plate or flask for longer than 5 days. Generally, cells are grown on plate for 5-7 days and sub-cultured into a new plate. Cells grown on a plate more than 5-7 days would became over confluent which lead to cell death. This could be the reason why Campisi *et al*. (2010) reported increase in DNA fragmentation.

Astrocytes communicate with their adjacent cells by secreting trophic factors such as BDNF. They are important in supporting neurotransmission and in promoting oligodendrocytes regeneration. BDNF, a member of neurotrophic factor family, was shown in

promoting survival and growth actions on neurons [21]. Downregulation of BDNF expression has been reported in a number of neurological disorders [22] and other conditions such as obesity [23]. Therefore in here, we aimed to investigate the effect of RF-EMF on BDNF expression of normal human astrocytes. However we did not observed significant effects of RF-EMF all the time points except for 1.8 GHz at 48 hr of exposure.

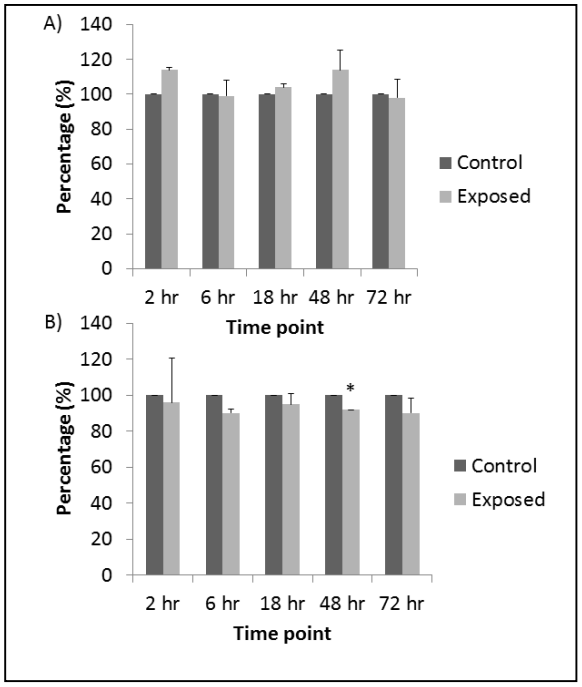

Figure 3: Expression of BDNF protein in human astrocytes exposed to 900 MHz (A) and 1.8 GHz (B) radiofrequency EMF from 2 hr to 72 hr. (*, indicated significant difference at $p < 0.001$ compared to control cells, n = 3 independent experiments).

Recently, Jadidi *et al*. (2016) reported that low EMF leads to increase of BDNF expression in Parkinson's disease rat models [24]. To date, no studies have reported any effects of RF-EMF on BDNF protein expression in normal human astrocytes. Hence, this study was the first to suggest that 1.8 GHz exposure for 48 hr could downregulate BDNF protein expression in human astrocytes.

In spite of numerous reports on the effects of EMF on cells, researchers reported conflicting results. Some researcher demonstrated positive effects of EMFs on cell proliferation [25,26]. In contrast, some researchers reported no effects of EMF on cell proliferation [19]. These divergences could be due to different laboratory setup and physiological responses (cell type dependent). In this study, we can conclude that RF-EMF at frequency 900 MHz and 1.8 GHz affect the proliferation rate of cells but no enough to cause apoptosis. Meanwhile, 48 hr of 1.8 GHz exposure could affect the normal level of BDNF protein expression in normal human astrocytes.

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