Assessing the effect of selenium on cyclin D1 level and nuclear factor kappa b activity in NIH/3T3 fibroblast cells at 2100 MHz electromagnetic field exposure

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Abstract

**Purpose:** Although there are various studies on the biological effects of the 2100 MHz electromagnetic field (EMF), which is among the mobile phone frequencies, there are conflicting results in the literature on this subject.

**Materials and Methods:** The present study was designed to assess the effect of 200 nM selenium (Se) on cell viability% [trypan blue], cell cycle biomarker [cyclin D1] and the transcription factor [nuclear factor kappa b (NF-κB)] in NIH/3T3 fibroblast cells exposed to 2100 MHz mobile phone frequency. Cells were divided to following groups: Control, sham control, 2100 MHz EMF, and 200 nM Se + 2100 MHz EMF for 2 h. And we measure cell viability%, cyclin D1 level and NF-κB activity.

**Results and Conclusions:** While of cell viability% decreased in 2100 MHz EMF group compared to control group, cell viability% increased in 200 nM Se + 2100 MHz EMF group compared to 2100 MHz EMF. It was also shown that while cyclin D1 level and NF-κB activity increased significantly in the 2100 MHz EMF group compared to the control group, the same parameters decreased in the 200 nM Se + 2100 MHz EMF group compared to the 2100 MHz EMF group. Although future studies will be required to investigate the biological effects of EMF emitted by mobile phones, our results provide insight into the molecular mechanisms underlying selenium's protective function and efficacy against 2100 MHz EMF exposure.

1. Introduction

The frequencies used by mobile phones range from 800 to 3500 MHz, while those described as 3G operate at a frequency of 2100 MHz. While the use of 4G is becoming more prevalent, the effects of the bandwidth of new generation technologies such as 5G and mobile phones on health are still unknown [1–3]. As technology develops, the use of mobile phones becomes more popular, resulting in further discussions on the possible detrimental effects on human health. It has been emphasized that long-term exposure to EMF leads to changes in biological systems through thermal and non-thermal effects resulting from the transfer of energy to tissue. From a biophysical perspective related to the potential thermal effects, it has been reported that the vibration of electrical charges on the cell membrane surface may lead to impaired electrochemical balance and cell dysfunction due to external oscillation, although studies of this subject to date are controversial [3, 4]. Previous studies have reported that the EMF emitted by mobile phones may have adverse effects on the cardiovascular system, immune system, thyroid and hormone secretion, the reproductive system and malignant tumors. Furthermore, studies have shown that at a cellular level, EMF can cause cell division and growth, oxidative stress, apoptosis and DNA replication [5–7].

Cyclins, which play an important role in cell cycle regulation, are a family of proteins that act by binding to cyclin-dependent kinases. D-cyclins (D1, D2, and D3) from the cyclin family bind and activate cyclin-dependent kinase-4 and 6 (CDK4/6) in the G1 phase of the cell cycle and form the cyclin D-CDK4 complex. This complex plays a critical role in the cell cycle by phosphorylating the retinoblastoma protein
Phosphorylation of RB is a molecular switch for the cell cycle, in which the hypophosphorylated RB forms a tight complex with the transcription factor E2F, inhibiting the replication of cells. Phosphorylation of RB, in turn, dissociates the complex and eliminates the transcriptional activity barrier on E2F. It has been reported that the dysregulation of cell cycle control by D-cyclins plays a role in the pathogenesis of various diseases, and that the dysregulation of the activities of these proteins may lead to increased cell proliferation and/or cancer [8–10].

There are many hormones and growth factors that affect cell growth and modify the activity of cyclins through signal transduction. NF-κB is a transcription factor that is involved in such cellular processes as inflammatory and immune cell response, cell cycle regulation, differentiation and protection against apoptosis. Factors such as bacterial endotoxin, phorbol esters, ultraviolet radiation, oxidants, viral proteins and double-stranded RNA activate NF-κB, which has been reported to be effective in controlling cell cycle and cyclin D1 by increasing the activity of cyclin D1 and cyclin D1 kinase holoenzyme complex [9, 11, 12].

In the human metabolism, enzymatic and non-enzymatic antioxidant systems eliminate the harmful effects of free radicals. Among these antioxidants, Se protects cells against oxidative damage by optimizing the activity of glutathione peroxidase and thioredoxin reductase, as well as some other selenoproteins. Recent experimental studies have reported that Se plays an important role in the cell cycle and apoptosis as a micronutrient and chemopreventive agent, depending on the chemical form and dose; however, the mechanism of action has not been fully clarified [5, 13, 14].

The present study assesses cell viability, cyclin D1 levels, and nuclear factor-kappa b activity in an investigation of the effect of selenium on NIH/3T3 fibroblast cells exposed to a 2100 MHz mobile phone signal.

2. Materials And Method

2.1. Experimental system

The experiment was set up in an anechoic chamber, where a signal generator (PSG Analog Signal Generator, Agilent Technologies E8257 D 250kHz-20GHz), power supply (Agilent 87421) and amplifier (Microwave System Amplifier, Agilent 5-26.5Ghz 83017 A) were used to conduct a 2100 MHz EMF exposure. The measurement, using the EMF probe (Broad Band Field Meter, Narda Safety Test Solutions EF0391 (NBB) E-Field Probe 100kHz-3GHz NBM550)) revealed an electric field of 12.18 ± 1.06 V/m, an RF output power of -18.50 dBm and SAR value 2 W/kg. To prevent the effects of any potential external or internal signals on study results, an EMF exposure cabinet with an aluminum-coated outer surface and an inner surface containing EMF absorbing material was designed. The humidity (90–95%) and temperature (37°C) of the system checks were recorded, and the experiment assembly was prepared. Then cells in the EMF group were placed in an anechoic chamber at equal distances and exposed to 2100 MHz EMF for 2 hours. The homogeneity of the EMF was checked by measurements. The experimental
setup and details are included in our previous study [15]. The EMF exposure system was designed in our laboratory, and the equipment was supplied by the Department of Electrical and Electronics, Electronics and Communication Engineering, Istanbul Technical University, Istanbul, Turkey.

2.2. Reagents and antibodies

All of the consumables (Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), phosphate-buffered saline (PBS), Trypsin-EDTA, and 1% penicillin-streptomycin) used in the cell culture experiments were purchased from Wisent, and sodium selenite and trypan blue have been acquired from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The nuclear and cytoplasmic extraction kit (Cayman-Item No: 10009277), NF-κB (p65) transcription factor assay Kit (Cayman-Item No: 10007879) and Ccnd1 (Aviva Systems Biology, OKEH03154) ELISA kit were supplied commercially.

2.3. Cell culture and selenium supplementation

The NIH/3T3 cell line (American Type Culture Collection (ATCC)) was incubated in 89% DMEM, 9% heat-inactivated FBS and a 1% penicillin-streptomycin antibiotic solution in a CO₂ incubator at 37°C, 95% humidity and with 5% CO₂. After the cells reached adequate confluency (80%), 1x10⁶ cells/T25 flask were seeded in each experimental group to form control, sham control, 2100 MHz EMF and 200 nM Se + 2100 MHz EMF groups. After the conclusion of the experiment, the adherent cells in all experimental groups were detached from the culture vessel and centrifuged at 300xg for 5 min. The supernatant was removed and cold 1XPBS was added to the pellet, which was stored at -80°C until analysis.

2.4. Cell viability analysis using trypan blue

Cell viability was analyzed using the trypan blue method, and the results were calculated using the formula: \[ \text{Cell viability\%} = \left( \frac{\text{Viable cell count}}{\text{Total cell count}} \right) \times 100 \].

2.5. Nuclear extraction preparation and NF-κB (p65) activity measurement

The nuclear extraction from the cells in 1XPBS was carried out according to the protocol provided with the Nuclear and Cytoplasmic Extraction Kit (Cayman-Item No: 10009277). NF-κB activity was measured in the nuclear extracts obtained from all experimental groups using an NF-κB (p65) Transcription Factor Assay Kit (Cayman-Item No: 10007879) in accordance with the kit protocol. Primary and secondary antibodies were added to each well of the oligonucleotide-coated 96-well plate included in the kit, which was then incubated. The optical density (OD) of the color change from blue to dark blue and then from blue to yellow was measured in an ELISA microplate reader (Multiskan GO-Thermo) at 450 nm within 5 min after adding 100 µl of the stop solution to the plate wells.

2.6. Measurement of cyclin D1 level

Cyclin D1 levels in the nuclear extracts were measured according to the protocol provided with the Cyclin D1 Elisa Kit (Aviva Systems Biology, OKEH03154). Samples were placed into each well of the 96-well plate included with the kit, which was then sealed using a cover sheet and incubated at 37°C for two
hours. After incubation, appropriate conjugates were added and the plate was incubated for one hour each at 37°C. The substrate specified in the procedure was added and the plate was incubated again for 30 min at 37°C without light. After these procedures, the optical density (OD) was measured by an ELISA microplate reader at 450 nm within 5 min of the color change of the wells from blue to dark blue, and then from blue to yellow upon the addition of 50 µL of the stop solution included in the kit. The results were calculated using the formula: \( \text{RelativeOD}_{450} = \text{WellOD}_{450} - \text{MeanBlankOD}_{450} \).

### 2.7. Statistical analysis

The study data were assessed using IBM SPSS Statistics for Windows (Version 20.1. Armonk, NY: IBM Corp.). The normality of the data was assessed with a Shapiro-Wilk test, after which a One-Way ANOVA was used to compare normally distributed data, while a Kruskal-Wallis test, one of the non-parametric tests, was used for the assessment of non-normally distributed data. The results were presented as mean ± standard deviation \((M \pm SD)\) and the statistical significance level was set at \(p < 0.05\).

### 3. Results

#### 3.1. Se stimulates the cell viability of NIH/3T3 fibroblasts exposed to 2100 MHz EMF

Cell viability % was decreased in the group exposed to 2100 MHz EMF when compared to the control group \((p < 0.05)\), and was increased in the group exposed to 2100 MHz EMF after incubation with 200 nM of Se compared to the group exposed to 2100 MHz EMF alone \((p < 0.05)\) (Fig. 1).

#### 3.2. Assessing the effect of Se on cyclin D1 levels of NIH/3T3 fibroblasts exposed to 2100 MHz EMF

The effect of Se on cyclin D1 level was evaluated by analysis of cyclin D1 level in NIH/3T3 cells exposed to EMF after Se supplementation. The cyclin D1 level of NIH/3T3 fibroblasts did not change significantly in the sham control group when compared to the control group, while there was a statistically significant increase \((p < 0.001)\) in the 2100 MHz EMF-exposed group. In the group exposed to 2100 EMF exposure after incubation with 200 nM of Se, the level of cyclin D1 was decreased significantly when compared to the 2100 MHz EMF-exposed group \((p < 0.05)\) (Fig. 2).

#### 3.3. NF-κB (p65) activity: Se protects NIH/3T3 cells against the effects of exposure to 2100 MHz EMF

Our results revealed the effect of Se supplementation on NF-κB activity in the NIH/3T3 fibroblasts exposed to 2100 EMF, as seen in Fig. 3. NF-κB activity saw no significant change in the sham control group when compared to the control group, while there was a significant \((p < 0.001)\) increase in the 2100 MHz EMF-exposed group when compared to the control group. NF-κB activity was statistically decreased in the group exposed to 2100 MHz EMF after incubation with 200 nM of Se when compared to the group exposed to 2100 MHz EMF alone \((p < 0.05)\).
4. Discussion

Recent reports have suggested that the EMF emitted by mobile phones affects cell division, oxidative stress and apoptosis mechanisms. Previous studies of cancer treatments have examined several compounds and mechanisms and their effects on cell proliferation and oxidative stress, although there is still a lack of clarity in this area [5–7].

In the present study we analyzed cell viability, cyclin D1 level which play a key role in cell cycle control and NF-κB activity to investigate the effect of Se on NIH/3T3 fibroblasts exposed to 2100 MHz EMF.

Previous studies involving people exposed to mobile phones, experimental animals, and normal and different cancer cell types have reported that EMF may produce significant effects through proliferation, apoptosis, DNA damage and oxidative stress, depending particularly on the duration of exposure [3, 16–22]. Yavas et al. reported an increase in total antioxidant, oxidant capacity and oxidative stress index in rats exposed to 2100 MHz RF-EMF [3]. Alkis et al. reported increased oxidative DNA damage and lipid peroxidation in the brain tissue of rats exposed to EMF at different frequencies (900, 1800 and 2100 MHz) [1]. Gorski et al. reported a decreased cell viability of human fibroblasts exposed to RF-EMF [17], while another study reported that 1800 MHz EMF increased cell proliferation and migration in Balb/c-3T3 cells [23]. Hou et al. reported apoptosis to be induced in NIH/3T3 cells through an increased level of reactive oxigen species resulting from an 1800 MHz electromagnetic field [21]. Park et al. reported that treatment with Se increased proliferation and migration in 3T3-L1 cells [24]. In a previous study of the human embryonic kidney cell line (HEK293 cell line), we found that exposure to 2.45 GHz EMF led to an increase in the level of lipid peroxidation and decreased antioxidant capacity. Our study further revealed that EMF exposure increased apoptosis and caspase-3 levels and decreased Bcl-2 levels, and that selenium exhibited a protective effect in groups treated with 100–200 nM Se, and had an antagonistic effect on EMF-induced cellular processes [13].

The present study, concurring with the results reported in literature, found that cell viability decreased in the 2100 MHz EMF-exposed NIH/3T3 fibroblasts due to the EMF effect, while viability increased in cells supplemented with Se. These results indicate that Se induced the active cell viability of the NIH/3T3 fibroblasts.

As our data suggest, 2100 MHz EMF exposure induced cyclin D1 level and NF-κB activity in the NIH/3T3 fibroblasts, while Se supplementation reduced the effects of EMF exposure. Changes in cyclin D1 levels may result in a dysregulation of cell-cycle control, leading to increased proliferation and cancer. Our study reveals that the cyclin D1 levels of the cell increase when exposed to 2100 MHz EMF, and the regulation of this situation with Se supplementation is important for cell division and cycle control. Furthermore, our study showed that NF-κB activity, which plays an important role in gene activation, cell growth, development and apoptosis, increased in parallel with cyclin D1 level at 2100 MHz EMF exposure, which was regulated by Se supplementation. Understanding the molecular mechanism of the cell cycle regulation and checkpoint abnormalities in cancer, and the manipulation of these control mechanisms, offers insights into potential therapeutic strategies.
Despite the need for future studies to explore the biological effects of the EMF emitted by mobile phones, our results provide insight into the molecular mechanisms underlying the protective function and efficacy of Se against exposure to 2100 MHz EMF. It is believed that the use of mobile phones, which can have various biological effects depending on the exposure time, may be a risk factor for cancer, although antioxidant supplements such as Se can be used as protective agents against the potential effects of the electromagnetic field emitted by mobile phones.

Declarations

Acknowledgement

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Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

There is no conflict interest in the study.

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References


**Figures**
Figure 1

Effect of Se on cell viability% in NIH/3T3 fibroblast cells exposed to 2100 MHz EMF (n=6). The results are expressed as the mean ± SD. The differences are shown different letters. a; versus to control, b; versus to 2100 MHz EMF group. *p<0.05; **p<0.01; ***p<0.001.
Figure 2

Effect of Se on Ccnd1 level in NIH/3T3 fibroblast cells exposed to 2100 MHz EMF (n=6). *The results are expressed as the mean ± SD. The differences are shown different letters. a; versus to control, b; versus to 2100 MHz EMF group. *p<0.05; **p<0.01; ***p<0.001.

Figure 3

Effect of Se on NF κB (p65) activity (OD450nm) in NIH/3T3 fibroblast cells exposed to 2100 MHz EMF (n=6). *The results are expressed as the mean ± SD. The differences are shown different letters. a; versus to control, b; versus to 2100 MHz EMF group. *p<0.05; **p<0.01; ***p<0.001.