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ORIGINAL: The Role of Vitamin E and Sodium Selenite Supplementation on the Apoptosis of Renal Cells from Mice under the Electromagnetic Field Exposure

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ABSTRACT

Introduction: By increasing the oxidative stress and the level of reactive oxygen species (ROS), electromagnetic field (EMF) is potentially able to damage body tissues, especially renal cells. Therefore, antioxidants by neutralizing the ROS may decrease the harmful effects caused by EMF. The aim of this study was to evaluate the effects of vitamin E and sodium selenite on the apoptosis of renal cells in mice under EMF exposure.

Material and Methods: Forty-eight mice, weighing between 24-28 g, were under EMF exposure for 4 hours a day during 2 months. Mice were categorized into 6 groups, containing 6 mice in each group, and were treated with vitamin E or sodium selenite or both. The control group received nothing. To evaluated the apoptosis rate, mice were sacrificed through cervical dislocation and the right kidney was extracted in the end of intervention period. Apoptosis rate was assessed by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining.

Results: It was detected that apoptosis was significantly increased in the EMF exposed mice. Treatment with vitamin E resulted in significantly decreased apoptotic cells in the EMF exposed mice. However, sodium selenite was unable to significantly decrees the apoptosis in the EMF exposed mice. Combinational therapy of vitamin E and sodium selenite resulted in more decreased apoptotic cells in comparison to the mice receiving only the vitamin E.

Conclusion: Combinational therapy of vitamin E and sodium selenite has beneficial effects in reducing the harmful effects of EMF on renal cells.

Introduction

Electromagnetic wave is generated through the sources of the radio frequency and almost all the electronic devices used in our daily life produces electromagnetic field (EMF) (1). There is evidence indicating that the biological systems and body cells react to a wide range of EMF. On the other side, a large proportion of the effects due to EMF exposure has been shown to be tolerated by cells regardless of clear detectable adverse effects (2). Nonetheless, epidemiological surveys have reported that either residential or occupational EMF exposure may lead to an increased risk of

malignancies, such as leukemia, breast and brain cancers (3, 4). Moreover, studies have disclosed a remarkable turbulence in the cellular metabolism, including proteins, carbohydrates, and lipids, which are manifested through modified blood glucose levels, increased glycolysis level, and intensified glycogenolysis in the various cells (5). Additionally, altered enzyme level and function, cell signaling, protein production, and gene expression, have been attributed to be stem from EMF exposure (6). It was demonstrated that exposing the cells to extremely low-frequency pulsed electro-magnetic fields (ELF-PEMFs) resulted in an increased oxidative stress in mammalian cultured cells (7), in chick embryos (8), and human erythrocytes (9). It has been suggested that the increased oxidative stress mediates an oxidative DNA damage and lipid peroxidation (10) that finally culminate in cell death (11, 12).

Vitamin E is the most frequently and potent radical-scavenging antioxidant. It is soluble in lipid and has antioxidant properties in the biological membranes and lipoproteins. Vitamins E inhibits the oxidation of low-density lipoprotein (LDL). During the scavenging the peroxyl radicals, vitamin E is converted into vitamin E radical, which is then oxidized into α-tocopheryl quinone. α-Tocopheryl quinone is the antioxidant form of the vitamin E (13). Sodium Selenite is the inorganic form of the selenium, which is a nutrient consumed in foods and have antioxidant properties. The antioxidative properties of selenium has been suggested to be mediated through the glutathione peroxidase family, which are antioxidant enzymes to reduce the ROS and maintaining the oxygen balance (14).

With respect to the harmful effects of EMF exposure on cells by generating ROS and oxidative stress, as we as the beneficial effects of Vitamin E and selenium on modulating the oxidative stress, this investigation intended to evaluate the effects of these supplements on apoptosis of renal cells in mice.

Methods

Mice

In this study, 48 mice with the weight range of of 24-28 g were used. The mice were prepared from the animal house of Tabriz University of Medical Sciences, Tabriz, Iran and kept in a standard condition (temperature 24 ± 2 °C, with a dark/light cycle of 12 hours). The whole process of working with animals was carried out in accordance with the protocol of the Ethics Committee of Tabriz University of Medical Sciences. After adaptation of mice with the condition of the animal house, they were randomly categorized into six groups, containing eight mice in each group. The treatment protocol of mice was as following: G1; control mice without any intervention. G2; mice with EMF exposure and without any intervention. G3; Mice with vitamin E and sodium selenite treatment without EMF exposure. G4; EMF-exposed mice with vitamin E treatment. G5; EMF-exposed mice with sodium selenite treatment. And G6; EMF-exposed mice with vitamin E and sodium selenite treatment.

Electromagnetic radiation

Electromagnetic wave (3 Mega Tesla and 50 Hertz) was radiated for 4 hours per day for 2 months using. Vitamin E (1.35 mg/kg body weight) and sodium selenite (0.1 mg/kg body weight) were administrated by a gavage daily for two months. After two months, the mice were sacrificed by cervical dislocation and their right kidneys were removed. The kidneys were embedded in paraffin, and finally, 5 µm sections were prepared for apoptosis evaluation.

Statistical analysis

The cortex of kidneys was stained using the Roche In Situ Cell Death kit (Detection Kit, POD, Germany). After removing paraphing, samples were placed in xylene for 10 mins. Lams were washed with phosphate buffer saline (PBS), and then were incubated with proteinase K for 20 mins in 37 °C. after that, tissues were permeabilized and then washed by PBS. Then, 50 µl of TUNEL solution was

added on each sample, which then was incubated for 1 hour in 37 °C. After washing, the tissues were scanned by Zeiss LSM 5 fluorescent microscope to count the apoptotic cells. The bright green spots were representing the apoptotic cells. The nucleus of cells was stained by 4′,6-diamidino-2-phenylindole (DAPI), which resulted in blue color dots under microscope. To stain the positive controls, both TUNEL-Enzyme solution and TUNEL-Label solution with the proportion of 1:9 was used. However, only TUNEL-Label solution was used to stain the negative controls.

Statistical analysis

Statistical analysis and plotting were conducted by GraphPad Prism v.7 (GraphPad Software, La Jolla, California, USA). The normality of data distribution was conducted by Kolmogorov-Smirnov test. Then, independent sample t-test was used to compare the number of the apoptotic cells between groups. Data were presented as Mean ± standard deviation (SD) and a P less that 0.05 was considered as statistically different comparison.

Results

Figure 1 demonstrates the fluorescent microscopic images of the apoptotic cells. It was observed that EMF exposure resulted in significantly increased apoptotic renal cells in comparison to the mice without EMF exposure (P = 0.021; ***Figure 2***). Moreover, in mice receiving vitamin E and sodium selenite without EMF exposure, the rate of apoptotic cells was significantly lower than the EMF exposed mice (P = 0.034). In the EMF exposed mice that were treated with vitamin E, the number of apoptotic cells were significantly lower (P = 0.042). However, sodium selenite was unable to inhibit apoptosis in EMF exposed mice and it was same as the EMF exposed mice receiving nothing (P = 0.067). Finally, in EMF exposed mice that were administered with both vitamin E and sodium selenite, the count of the apoptotic cells was significantly lower in comparison to the EMF exposed mice receiving nothing (P = 0.039). Combinational therapy of vitamin E and sodium selenite led to more decreased apoptotic cells in comparison to vitamin E only (P = 0.046).

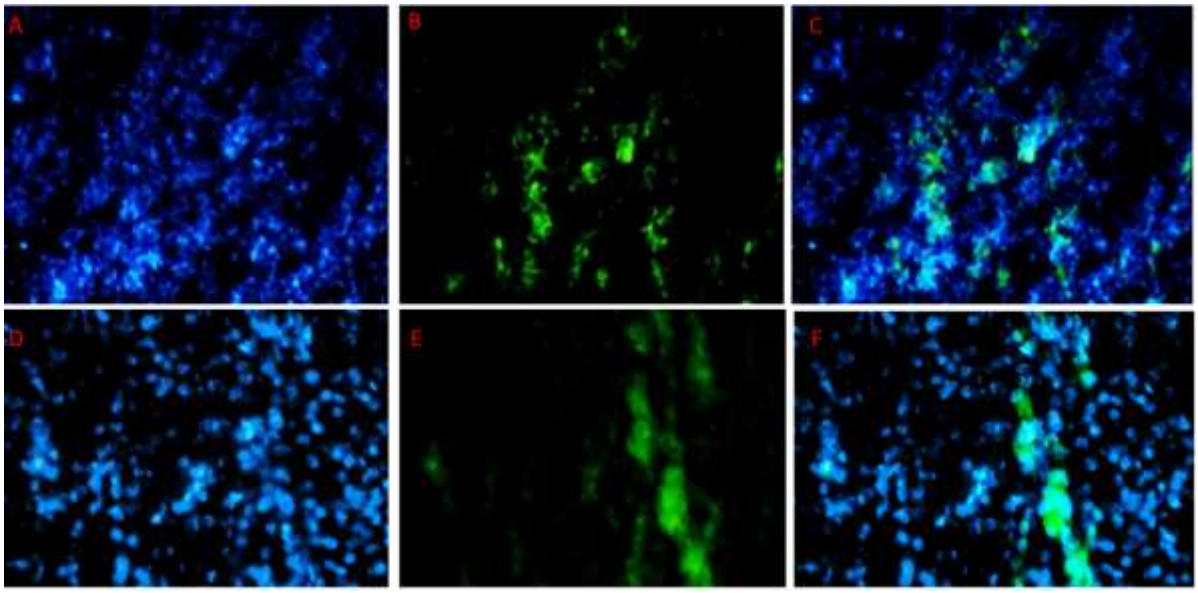

Figure 1. TUNEL staining of the kidney tissues obtained from mice under EMF exposure. After 2 months of exposure, kidneys were extracted and stained. The fluorescent microscope was used to count the apoptosed cells. The nucleus of cells was stained by 4′,6-diamidino-2-phenylindole (DAPI), which resulted in blue color dots under microscope (A; 200x magnitude and D; 400x magnitude). Apoptotic cells were appeared as green (B; 200x magnitude and E; 400x magnitude). C and F images demonstrate merged staining for the nucleus and apoptotic cell with 200x and 400x magnitude, respectively.

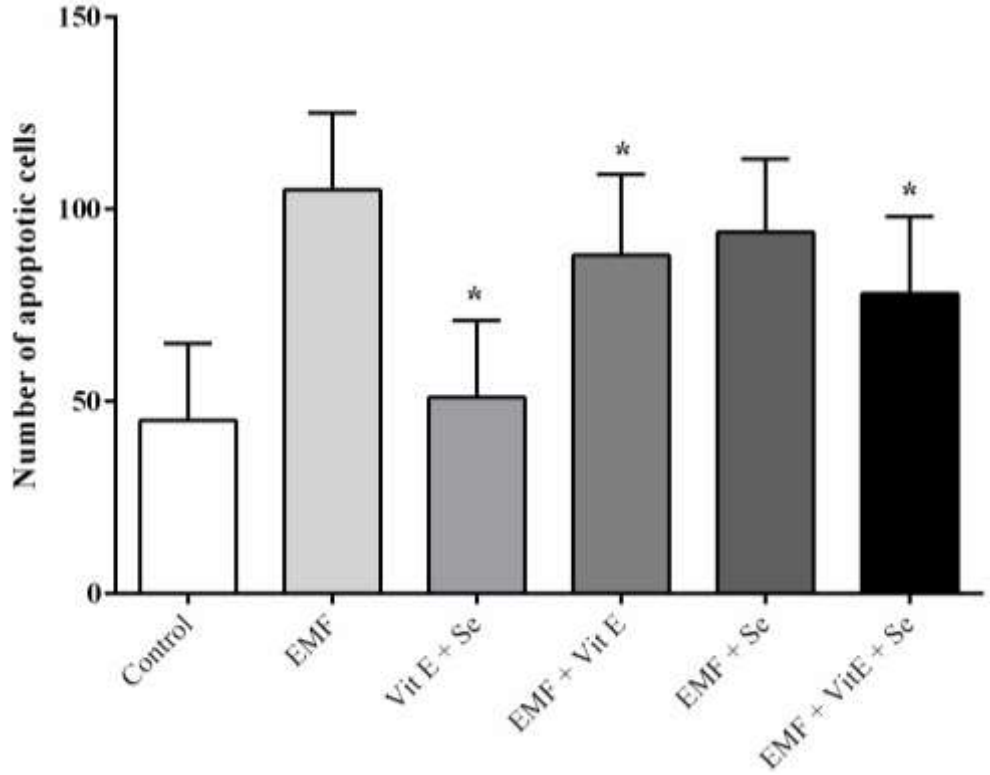

Figure 2. Bar graphs demonstrate the number of the apoptotic cells in different groups of mice (* indicates a P < 0.05).

Discussion

In the current study, we intended to evaluate the therapeutic effects of the vitamin E and sodium selenite on reducing the apoptosis rate in renal cells from mice under EMF exposure. Experiments demonstrated that vitamin E, but not sodium selenite, was able to inhibit apoptosis in the EMF exposed mice. Combination of vitamin E and sodium selenite resulted in more decreased apoptosis rate in the EMF exposed mice in comparison to the mice that received only vitamin D.

Mobile phones and their stations generate electromagnetic radiation that is composed of electrical and magnetic parts (15). The electrical part is generated by a voltage gradient. However, the magnetic part is produced due to flow of electric current. Because of penetration into living tissues and cells more easily, the magnetic field is more harmful than the electric field (16). The electric and magnetic fields are separated from each other at the very low frequencies. However, at higher frequencies, the electric and magnetic fields are combined, and they are called electromagnetic fields (17). It has been reported that the EMF are dangerous to the public health (18) and more than 3 billion individuals worldwide are exposed to EMF daily (19). EMF can penetrate into several tissues and cells, particularly kidneys and liver (20).

The biological impressions of EMF can be classified into the two thermal and non-thermal groups. The thermal effects are attributed to local generation of heat. However, the non-thermal effects are not directly attributed to the heat and are associated to other changes generated in the cells (21). EMF can penetrate into the body and impress all cell types by changing the potential of cell membrane as well as the ion concentrations. These modifications, in turn, may impress the biochemical processes within the cells (22). EMF can confer a turbulence in intracellular signaling pathways by altering the Ca+2 permeability through the cell membranes and, thus, the calcium levels within the cells (23). Moreover, by enhancing the levels of free radicals and increasing lipid peroxidation, and by reducing the function of the antioxidants and promoting the oxidative

stress, EMF interferes massively with the normal biological activities of cells (16, 24). The vitamin E and sodium selenite have been attributed with antioxidative effects through different mechanisms and, therefore, may be beneficial in reducing the harmful effects of EMF. In a study, the effects of vitamin E and C were evaluated in improving the endometrial tissue impairments in rats that was induced by 900 MHz mobile phone. It was observed that the levels of nitric oxide (NO, which is an oxidant compound) and malondialdehyde (MDA, which is an index of lipid peroxidation) were enhanced in EMF exposed rats, which were reduced by vitamins E and C. Moreover, treating with vitamins E and C resulted in increased activity of glutathione peroxidase enzyme (an anti-oxidant enzyme), which was decreased due to EMF exposure. Moreover, the apoptosis of the endometrial surface epithelial and glandular cells and the stromal cells was reduced in the vitamins E and C treated group (25). It seems that vitamin E as well as sodium selenite can increase the production of the antioxidant enzymes and, thus, mediate their beneficial effects (26). Actually, oxidative stress has been associated with an alteration in the apoptosis related genes, such as Bax and Bcl2, and therefore increase apoptosis of cells (27, 28).

Our experiments demonstrated beneficial effects of vitamin E in decreasing the apoptosis of renal cells due to EMF exposure. Nonetheless, this effect was not observed in mice treated with sodium selenite. Studies have indicated that vitamin E and sodium selenite have synergistic effects. Sodium selenite, as a co-factor for glutathione peroxidase enzyme, enhances the effect of vitamin E in the intracellular antioxidant system (29). Therefore, further studies are still needed to answer the question why sodium selenite could not decrease the apoptosis rate in the EMF exposed mice. Interestingly, our experiments demonstrated more decreased apoptosis rate in the vitamin E and sodium selenite treated mice in comparison to the mice that received only vitamin E.

Conclusion

In consideration of all, the purpose of this study was to assess the positive effects of the vitamin E and sodium selenite on decreasing the apoptosis rate in renal cells from mice under EMF exposure and revealed that vitamin E, but not sodium selenite, decreased the apoptosis in the EMF exposed mice. Therefore, it is suggested to consume products containing these compounds to reduce the effects of EMF that people are daily exposed with it from various sources like cell phones and televisions. However, it is suggested to conduct further molecular studies to disclose the exact mechanism of apoptosis reduction by vitamin E and sodium selenite.

Ethical standards statement

All the procedures were in accordance with the guidelines for care and use of laboratory animals, adopted by the Ethics Committee of Tabriz University of Medical Sciences.

Conflicts of interest

The authors have no conflict of interest to declare.

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