Protecting effect of vitamin E against chromosomal damage induced by an extremely low-frequency electromagnetic field in murine bone marrow erythrocytes

Abstract

Living organisms including human are exposed to an electromagnetic field from natural and industrial sources. Genotoxic effects of electromagnetic fields have been reported by several studies. Vitamin E is a lipid-soluble antioxidant that plays an important role in maintaining the integrity and function of cell membranes, nervous system, and reproductive system, among others, by functioning as a defence against oxidative damage. In this research, the protective effects of vitamin E against the chromosomal damage induced by extremely low-frequency electromagnetic field (ELEM) on bone marrow erythrocytes of adult male BALB/c mouse have been studied. Adult male BALB/c mice were intraperitoneally injected with vitamin E, with the dose of 200 mg/kg for 8 days and then exposed to ELEM with the intensity of 50Hz for 4 hours for 4 consecutive days. The mice were dissected and micronucleus assay was performed on the polychromatic erythrocytes (PCE) of their bone marrow. Results show that ELEM could increase the chromosomal damage judged by the higher frequency of MN compared to control. Results also revealed that the frequency of micronucleated polychromatic erythrocytes (MNPCE) had substantially decreased ($p<0.00\ 1$) in vitamin E- treated mice compared to untreated. Data suggest the protecting effect of vitamin E in bone marrow cells of Balb/C mice against the ELEM.

Keywords: electromagnetic fields, vitamin E, erythrocyte, BALB/c, micronucleus.

Introduction

Development and industrialization of societies have led to the utilization of electricity more than ever. In recent years, due to the abundant use of devices generating electromagnetic waves, the study of their biological effects on human and other living organisms' health has become more and more important (1). These waves are generated by different devices and appliances, such as

refrigerator, television, microwave, computer displays, printers, and halogen lamps and so on. Several studies have been performed on the analysis of the effect of waves at the molecular, cellular levels and also on the field of biophysics and cancer protection (2). Many studies have proven that electromagnetic waves affect living organisms function at the cellular and molecular level (3, 4, 5 & 6). Because of its proven harmful effects on living organisms, attempts are being made to find out the solutions to reduce or prevent its unwanted biological effects (2, 3).

Also, there is evidence showing the relationship between exposure to electromagnetic fields and gene expression irregularities and damage to DNA molecule (7). Damage to this molecule is important because of its role in cell proliferation and viability, and also mutations and cancer.

Electromagnetic waves had genotoxic effects leading to chromosomal instability and increase of structural damage to chromosomes on peripheral blood of human and different cells of other mammals (8, 9).

Electromagnetic waves are shown to be a potent oxidative agent to the cells and living organisms. It can cause oxidative damage to DNA by increasing the extent of lipid peroxidation and the iron level while decreasing total antioxidant status, copper, and GSH values through overproduction of free radicals/reactive oxygen species (10). These oxidative changes may lead to the oncogenic transformation of the exposed cells.

Antioxidants can prevent the damage caused by free radicals and protect the macromolecules of the cells through their scavenging capabilities. One of these antioxidants is vitamin E, this vitamin is lipid-soluble and has an essential role in biological membranes, lipoproteins, nervous and reproductive systems. There are many reports about the function of this vitamin in preventing or improvement of the diseases, for example, its role in cardiovascular disease and ovarian cancer (11,12). This vitamin acts as the most important lipid-soluble antioxidant and prevents reproduction and dissemination of harmful free radicals in a biological system (13,14). The destructive effect of free radicals occurs in the absence of vitamin E and leads to peripheral nerve damage (13). Also, various studies have proven the role of vitamin E against induced genetic damage by genotoxic substances such as Cisplatin, beryllium, acrylamide (15,16)

Since antioxidant effects of vitamin E in inhibition of induced damage by electromagnetic waves with low frequencies have not been studied so far, the study was performed to investigate the protective effect of vitamin E against electromagnetic field with frequency of 50 Hz and intensity of 50 Gauss on mouse bone marrow cells (in vivo) using micronucleus assay.

Materials and Methods

Animals:

Protecting effect of vitamin E against electromagnetic waves were studied on polychromatic erythrocytes of bone marrow of adult Balb/C male mice, with the weight of about 30-25 grams(from Mashhad Razi Serum Institute) as an experimental model.

The mice were kept in natural conditions with about 60 – 70% humidity, 23 ±1 °C temperature and a photoperiod of 12 h light, 12 h darkness in polycarbonate cages. Food and water were also supplied Ad libitum.

Experimental procedure:

Forty mice were randomly divided into 4 groups, including:

Control: mice were kept in normal conditions

Test 1: mice injected (IP) with vitamin E with a dose of 200 mg/kg bw for 8 consecutive days

Test 2: mice in this group were exposed to electromagnetic wave using the device made at Azad Islamic University, Mashhad branch, with intensity of 50 Gauss for 4 days, 4 hours daily Experimental group: vitamin E was injected with dose of 200 mg/kg bw for 8 consecutive days and at 5^{th} day of injection, they were exposed to electromagnetic waves with intensity of 50 Gauss for 4 days, 4 hours daily.

Slide preparation:

All mice were anaesthetized with chloroform and were dissected. The bone marrow of two legs was removed by injection of 0.5 ml of fetal bovine serum (GIBCO) and was collected in a test tube. The tube containing the suspension was centrifuged (Kokusan, Japan) for 10 minutes at 900 rpm.

The cell palette resuspended in 0.5 ml of FBS. Four to five drops of cell suspension were placed on a slide. Air-dried slides were fixed by absolute Alcohol for 5 minutes. Staining was performed according to by Schmid using Migranvald and Gimsa (Merck, Germany)

Scoring:

In a well-prepared slide, polychromatic erythrocytes are clearly observed as pink; these cells have no nucleus. In these anucleated cells, micronucleus was visible as a small purple core in the cytoplasm. Were prepared 6 slides per treatment, in each slide, the number of MNPCE was counted in 1000 PCEs (Magnify x1000) and the mean score is compared with the mean score for the control group.

polychromatic erythrocytes lack a nucleus, which implies that the presence of micronuclei in their normally anucleated cytoplasm signifies encapsulated DNA fragments, a tell-tale indication of chromosome damage

Statistical Analysis

Data analysis was performed in SPSS and the statistical significance was determined using one-way analysis of variance and Tukey's multiple comparison tests. In all the analysis a 95% confidence level ($P<0.05$) was considered statistically significant. All the data were presented as the mean ± standard deviation (SD).

The ethics of animal handling and treatment were considered throughout the experiment.

Results

The mean frequency of MnPCE in all groups is presented in image1. Injection of vitamin E significantly decreases the MnPCE frequency compared to control.

However, the result revealed that the frequency of MnPCE was significantly higher in electromagnetic waves treated mice compared to control($P<0.001$). Treatment of the mice with

vitamin E four days before exposure to electromagnetic waves did reduce the frequency of MnPCE compared to electromagnetic waves treated mice (P<0.01) and brought it to the control level.

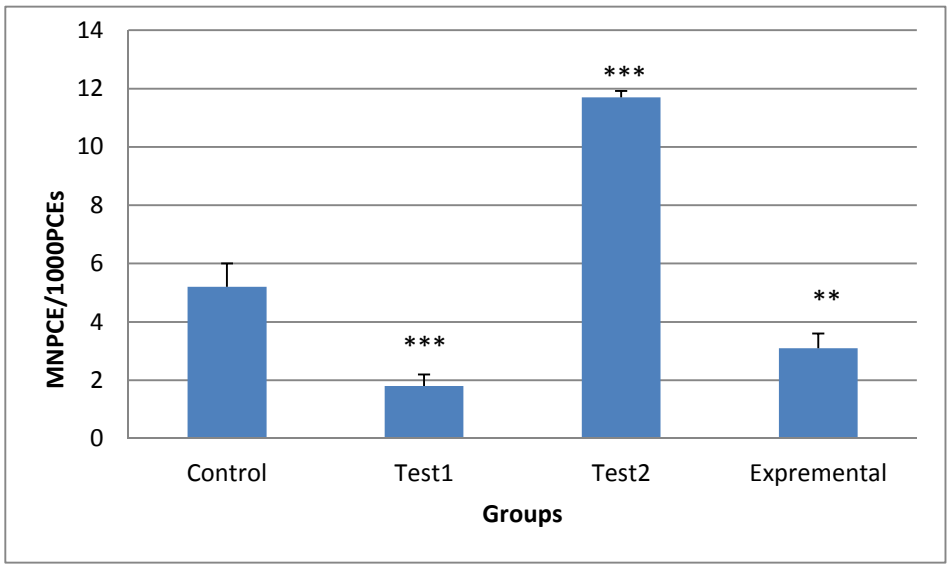

Figure1: The mean score of MnPCE in each group compared with the mean score for the control group. (**P<0.01, ***P<0.001)

Discussion

In this study, the genotoxic effect of extremely low electromagnetic fields was investigated on mice using micronucleus assay. This test is the most common, sensitive and quickly genotoxic test that shows chromosomal damages, including the loss or breakage of chromosomes. (17,18,19). It was first performed by Schmid in 1973 in the mice bone marrow erythrocytes (20,21,22).

Data of the present study revealed the genotoxic effect of the electromagnetic field in bone marrow polychromatic cells. Increase in the frequency of micronucleus in lymphocytes of the workers exposed to the electromagnetic field is an indication for its clastogenic effect (23) Exposure of the pregnant rats to electromagnetic field led to tissue and DNA damage in testis

of male newborn rats (24). Also, mice exposed to electromagnetic fields represented by tissue damage in their kidneys and testis caused by oxidative stress(25).

The result of this research is in accordance with the findings of several studies showing the relationship between exposure to the electromagnetic field and induction of DNA damage, such as chromosomal instability, increase of chromosomal breaks, sister chromatid exchange and increase in the frequency of micronucleus in mice or rats(26,27). Rats exposed to long treatment with 900 MHz electromagnetic fields exhibited a high frequency of micronucleus in Polychromatic erythrocytes(22).

There are a variety of experiments about the cause of damage by electromagnetic waves. Since the energy of electromagnetic waves isn't high enough to cause direct damage and cracking, it seems that fields affect indirectly. Some researchers believe that electromagnetic and radio waves affect by increasing the ROS reactive oxygen. Simko has proposed that these waves increase the free radicals level (28)

The clastogenic property of a magnetic field is due to its ability to induce oxidative stress in cells and reducing the total antioxidant status and glutathione value in the cells (25). Hence antioxidant treatment of the exposed cells to electromagnetic fields might reduce the clastogenic damage in those cells. In this experiment, we showed that treatment of the mice with vitamin E before exposure to the extremely low magnetic field can reduce the frequency of micronucleus in polychromatic erythrocytes of bone marrow.

It was observed the waves increased cracking in DNA when rats' brain cells were exposed on the microwave waves 2/45 GHz for 35 days (26). Mice of bulb C with age of 7 weeks were exposed to electric fields 3 Tesla for 24 hours and intensity of 4/7 Tesla for 24, 48 and 72 hours. The results showed that frequency of micronucleus is dependent on the radiation time and intensity of field (27).

Ruedger has also expressed that the effects of RF-EMF genotoxic can be through the production of free radicals and reaction with DNA improvement mechanism (28). Zmysolonyi has been reported

the formation of free radicals under the effect of electromagnetic waves is the main reason of destructive effects of these waves (29). So we can conclude that free radicals are the reason for production micronucleus. Eldamerdash with the study of mobile phone waves has shown that waves cause changes in the antioxidant activity of rats' blood and brain (30).

Awad also stated in his study that ROS has an important role in tissues exposed to waves emitted from mobile, and leads to damage to the antioxidant system (31). Erguder has reported the oxidative stress is the reason of damage to the samples exposed to waves emitted from the computer (32). Claycombe et al. have stated that oxygen free radicals as a Mutagen cause many cancers and create defects in the immune system (13).

Evaluation of the effect of electromagnetic waves and peritoneal injection of vitamin E showed that mean number of micronucleus is significantly decreased. Vitamins as an antioxidant are necessary for health. The effect of vitamin E on antioxidant system of liver, kidneys, and muscle of rabbit is positive (33).

Vitamin E is a fat-soluble vitamin and plays a role in lipoprotein metabolism and biological membranes and can induce or prevent of a gene or protein expression (10). The results of this 173research match with the reports of Ouanes, Robichova, Singha, and Gonzalez that all have mentioned anti genotoxicity role of vitamin E. Ouanes stated that vitamin E reduces the induced micronucleus by Zearalenone (34,35). Robichova has also reported that vitamin E decreases the damaging effects of N-nitrozomorfolin in human hepatic cells (36). Singha has improved the effects of genotoxic atrazine on rats' blood cells and liver with the effect of vitamin E (37). Dicamba is a genotoxic substance (SCE) which causes DNA damage with the production of free radicals. Gonzalez has stated that vitamin E improves the adverse effects of this substance (38).

It has been observed that antioxidants reduce the percentage of chromosomal elimination in chromosomal patients. Dusinska reported that antioxidants decrease the chromosomal damage (39). Using 300 mg/kg bw Vitamin E for 21 consecutive days decreases the number of induced micronucleus by Acrylamide in rats' bone marrow (40). Microelements have an interventional role on gene's function due to their role in the vital path and different reactions, and their lack impact on gene function (41). Consumption of vitamins leads to the stability of genome and reduction of

Micronucleus (42). Erguder has also suggested the consumption of vitamins and antioxidants for reducing the effects of the computer (32).

Claycombe also expressed that vitamin E as an antioxidant decreases chromosomal damage by preventing the formation of free radicals. In fact, antioxidants converted free radicals into harmless compounds that easily leave the body before they damage to any part of the body. Even antioxidants may improve previous damage (9). Results of this research do not match with Hsieh's experiments that reported the destructive effects of vitamin E. The reason of contradictory results can be the used dosage. He observed that vitamin with dose of 500 mg/kg has genotoxicity role, and antioxidants act as Peru-oxidation in high concentrations and create Autoimmune' damage (43). He has also stated that Vitamin E is a cancer promoter in high concentrations. The amount of damage by free radicals or under the effect of environmental factors and presence of other antioxidants can influence the effectiveness of this vitamin (44).

The study performed by Demsia reported that long-term radiation for 30 days, 3 hours per day increases micronucleus in polychromatic erythrocytes of rat's bone marrow three times in comparison with control group (45). Ruiz-Gomez's tests have shown that electromagnetic waves could act as a co-inductor of DNA damage (46).

This result is contradictory with some experiences. The experience of Vijayalaxmi has suggested that electromagnetic field with 42 GHz for 30 minutes does not significantly increase the number of micronucleus in polychromatic erythrocytes of mice with a race of BALB/c (47). Comparison of the effect of radio waves 1800 MHz on the number of micronuclei in rat cells exposed to the field for 5 days and 20 minutes daily with the control group showed no significant difference between two groups (48).

It seems that different results of various studies depend on changes in study conditions, especially frequency change, intensity and radiation duration, type of animals and type of magnetic field (constant or intermittent).

The findings of this study showed that vitamin E will reduce genotoxic effects by an electromagnetic field with low-frequency of 50 Hz in polychromatic erythrocytes of mice bone

marrow. It seems that the use of food sources of vitamin E is very important and significant for the persons living in the environment of electromagnetic waves.

Conclusion

Since electromagnetic field applications are increasing in human life, it is required to use effective and non-toxic compounds that can reduce genotoxicity induced by an electromagnetic field.

The results of the present study showed that extremely low-frequency electromagnetic field induce genotoxicity on bone marrow erythrocytes. Treatment with a known antioxidant, vitamin E has shown the protective effect of DNA damage.

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