ROLE OF VITAMIN E IN PREVENTION OF DAMAGES IN THE THYMUS INDUCED BY ELECTROMAGNETIC FIELD: ULTRASTRUCTURAL AND LIGHT MICROSCOPIC STUDIES

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Abstract

Twenty-four male Wistar rats were selected and divided into three groups (control, test group 1, test group 2). The test group 1 was exposed to EMF (50 Hz, 3 mT) 8 h a day, 6 d per week for 2 months. Test group 2 was exposed to EMF (50 Hz, 3 mT) 8 h a day, 6 d per week for 2 months but received orally 30 mg of vitamin E/d. Rats in the control group neither were exposed to electromagnetic field nor received vitamin E. At the end of the experiment, the rats were sacrificed, dissected, and samples from the thymus were taken and processed for light and electron microscopic studies. Forty microscopic fields from each group were randomly selected and studied. The data showed that in the thymus of test group 1, the population of cells in the cortex was decreased but the number of macrophages was increased. EM study showed that cellular nuclei were heterochromatic in comparison to control group. Test group 2 was similar to the control group. These findings indicate that immune system is weakened by electromagnetic field but vitamin E supplementation prevented above alteration.

Key words: rats, thymus, electromagnetic fields, vitamin E.

The wide-spread application of electromagnetic field (EMF) in everyday life has produced many concerns on health effects of daily environmental exposure to electromagnetic radiation. There are many types of equipments emitting such radiation, among them; some could have therapeutic application (1, 14, 17). EMF can affect cells via different mechanisms. For instance, electromagnetic radiations can cause a change in functional potential of the cytoplasmic membrane due to biochemical changes followed by a change in concentration of ions trafficking within the cell membrane (3). In addition, the physical reaction between EMF and chemical bonds among atoms could lead to formation of free radicals in the cells (5, 11, 13). There are many data showing that long time exposure to EMF has side effects on living organisms (9, 24). However, there are also studies indicating that electromagnetic field has no side effect on living organisms (8). There are several studies about the EMF effects on lymphatic organs but some of them have been focused on serum parameters like different enzymes. For instance, the study by Koyu et al. (11) demonstrated that when rats were exposed to EMF (900 MHz), activities of CAT, SOD, GSH-Px, and XO were changed. There are data showing that EMF exert their effect via formation of free radicals (5, 11, 13). Since vitamin E is a well known antioxidant (16) and its protective role in EMF–exposed animals was demonstrated (15), the aim of the presented study was to investigate the protective effect of vitamin E on long-term induced effects of EMF on the thymus in rats.

Material and Methods

Four-month-old male Wistar rats were used as laboratory models. The animals were kept in a humid atmosphere (65%-70%) at 25°C, with a 12 h time interval access to light and darkness during each day, and one week of aclimatisation was provided. All procedures performed on animals were approved by the local Animal Care and Use Committee. In order to generate electromagnetic field (EMF), a generator using 220 V and 50 Hz electricity, based on Helmholtz coil, and generating a field of 3 mT was applied.

The apparatus was designed to accommodate eight rats and a water circulator system preventing increase in animal internal temperature. The power system and frequency producer were checked by Tesla meter for an accurate and homogenous EMF performance. Additionally, the intensity of EMF generated by the apparatus was examined before and during the study using the apparatus-associated Tesla
meter. Initially, the animals were randomly divided into three groups: two test groups and one control group with eight rats in each group. The rats in control group received standard feed without being exposed to EMF. Test group 1 received their feed like the control group but was exposed to EMF (3 mT, 50 Hz) for 8 h a day, for a total period of 2 months. The test group 2, received 30 mg v.E supplements every day in their feed and was exposed to EMF (3 mT, 50 Hz) for 8 h a day, for a total period of 2 months. After the termination of experimental period, all rats were sacrificed and samples of the thymus were collected. The samples were fixed in 10% formalin for 48 h, processed in tissue processing device, and 5 µm thick paraffin sections were prepared. Out of many sections from each sample, the sections no. 2, 4, 6, 8, and 10 were chosen and stained with haematoxylin and eosin (HE). Finally, 40 microscopic fields from a similar number of slides in each group were randomly selected for cell count, photography, and light microscopic study. At the same time, five samples from each group were fixed in 2% glutaraldehyde and prepared for electron microscopy (EM) examination. The ultrathin sections were examined under LEO 906 TEM.

Statistical analysis. For statistical analysis of data, Student’s t-test was used. P<0.05 values were considered as significant.

Results

The results are as follows: in the control group, the cortex and medulla could be differentiated clearly (Fig. 1-A), the mean number of macrophages in the medulla of this group was 2.72 ±1.11. In test group 1, cellular nuclei were all heterochromatic and the border of the cortex and medulla was not clear (Fig. 1– B). The mean number of macrophages increased to 9.50 ±2.02. These parameters in test group 2 were similar to the control group (Fig. 1-C), and the mean number of macrophages was 3.21 ±1.10.

As it is clear from the data, there was a significant difference between the number of macrophages in control and test group 1 (P<0.05), but not between control and test group 2 (P>0.05).

The examination of the thymus with higher magnification revealed that nuclei in lymphatic cells were heterochromatic in the test group 1 (Fig. 2-A) in comparison to the control group (Fig. 2-B). However, in the test group 2, they were similar to the control group (Fig. 2-C). Additionally, test group 1 contained a lot of activated macrophages (Fig. 2-D).

The examination of blood-thymus barrier with higher magnification showed that this barrier in the test group 1 was irregular (especially in basal lamina that surrounded capillaries) (Fig. 2-E) in comparison to the control group (Fig. 2-F). Test group 2 was similar to the control group (Fig. 2-G).

Fig. 1-A: The thymus from control rat. Note sharply defined cortex (dark) and medulla (light). HE, 660x.

Fig. 1-B: The thymus from a rat exposed to EMF (test group 1). Note the effacing of cortex and medulla border. HE, 660x.

Fig. 1-C: The thymus from a rat that received vitamin E supplement during EMF exposure. Cortex and medulla are similar to control group. HE, 660x.
Fig. 2-A: A lymphocyte from the thymus of EMF-exposed rat (test group 1). Note heterochromatic nucleus. EM, 7,750x.

Fig. 2-B: A lymphocyte from the thymus of control rat. Note an euchromatic nucleus. EM, 7,000 x.

Fig. 2-C: A lymphocyte from the thymus of rat that received vitamin E during EMF-exposure. Note that nuclear condensation is similar to control group. EM, 6,000x.

Fig. 2-D: An active macrophage with numerous lysosomes in the thymus from EMF-exposed rat. EM, 2,784x.

Fig. 2-E: Vascular basement membrane in the thymus from rat exposed to EMF (test group 1). Note that the normal organisation of basal lamina disappeared. EM, 21,560x.

Fig. 2-F: Vascular basement membrane in the thymus from control rat. Note the structure of basement membrane as dark and light light lines. EM, 35,960x.
Discussion

In spite of large number of studies on the effect of EMF on living organisms (2, 9, 15, 19, 20), a protective role of vitamin E on the effect of EMF exposure has not been studied at ultrastructural level. Therefore, in the present study a protective role of the vitamin on the effect of EMF in the thymus was investigated. Our findings demonstrated a condensation of cell nuclei in experimental test group 1 indicating that EMF has a nuclear effect and could at least suppress cellular metabolism. These findings are well supported by previous studies showing that EMF has a nuclear effect (7, 24, 26). It is believed that EMF, by producing free radicals, could result in DNA damage (5, 11, 23, 24). If the detrimental effect of EMF is attributed to the production of free radicals, addition of vitamin E supplement prior to EMF exposure could prevent the nuclear effect of EMF. Accordingly, in our experiment in the test group 2, which received vitamin E along with exposure to EMF, nuclear condensation did not occur. This finding is in agreement with previous studies proposing that EMF induces its damages via formation of free radicals (5, 11, 13) and vitamin E as an antioxidant could neutralise detrimental effects of EMF (16). In another study, Factor et al. (6) demonstrated that vitamin E reduced chromosomal damage caused by increased reactive oxygen species (ROS), and also Wolf et al. (23) showed that EMF cause DNA damage through the action of free radical species that could be prevented by pre-treatment with an antioxidant. The vitamin E, beside its antioxidative properties, is an important factor for thymus activity; however, any proof concerning this matter was provided in this study.

Our studies also showed that in the control group, the border of cortex and medulla was clearly observable but in the EMF exposed group it was not. It is clear that cortex of the thymus is an active site of lymphatic cell proliferation and differentiation. It appears that the observed changes were due to the decreased cellular population caused by detrimental effect of EMF on proliferating cells in these areas. This finding is well supported by previous studies, as it was established that actively proliferating cells are more sensitive to environmental factors including EMF. Kaszuba et al. (10) showed that EMF-induced cellular mortality in cultured cells was increased by addition of phytohaemagglutinin as a mitogen, and Onodera et al. (18) found that detrimental effect on immune cells during cell division was more evident than on cells being in a non-dividing phase. However, the changes did not appear in animals that received vitamin E.

Regarding the increasing number of macrophages, it is clear that they are defensive cells. There are several reports showing that under stimulatory condition and tissue damage macrophages become more activated (4, 22, 26) and their number increases (12). On the other hand, Simko et al. (21) have shown that EMF resulted in significantly increased phagocytic activity of macrophages, which indicates that the number and activity of macrophages increased due to the increasing cellular damages.

In the test group 2, that received vitamin E supplementation before the exposure to electromagnetic field, no increase in the number of macrophages was observed.

It can be concluded that, in general, EMF has a suppressive effect on the immune system by affecting the population of lymphocytes and this effect could be prevented by vitamin E supplementation.

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References

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