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Changes in Oxygen Free Radicals Caused by an ELF Magnetic Field in Hepatic Tissue Homogenats of Ovariectomised Female Rats

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The aim of this study was to estimate the influence of long-lasting exposure to extremely low frequency (ELF) magnetic fields on amount of oxygen free radicals activity indicators in rat hepatic tissue homogenats in ovariectomized female rats. The experimental material consisted of 35 female Wistar rats: twenty seven animals bilaterally ovariectomized in the 6th month of age constituted the experimental group exposed to a magnetic field, and 8 unovariectomized rats made up the control group in which a sham-exposure was made. In the ovariectomized group, 10 rats were exposed to a rectangular magnetic field at a frequency of 5 Hz and magnetic flux density of 10 mT rms, 9 rats were exposed to a sinusoidal magnetic field at a frequency of 40 Hz and magnetic flux density of 10 mT rms and finally 8 animals were exposed to a sham-exposure of 1 hour a day for 40 days. In all animals the amount of oxygen free radicals activity indicators — some antioxidant enzymes and MDA in hepatic tissue homogenats was estimated. The amount of POX decreased significantly in both groups exposed to a magnetic field as compared to the sham-exposed group. No significant changes in CuZnSOD and GST tissue content was observed in both exposed groups as compared to the sham-exposed group and to the control group of non-ovariectomised rats. The amount of MDA in ovariectomised rats was insignificantly higher in comparison with the control non-ovariectomised group, while the exposure to a magnetic field caused a significant decrease in MDA content in both exposed groups as compared to the sham-exposed group. Results of the present

study have proved that ELF magnetic fields cause significant changes in some oxygen free radicals activity indicators content in hepatic tissue homogenats of ovariectomized rats.

Key words: oxygen free radicals activity indicators, hepatic tissue homogenats, ovariectomised female rats, extremely low frequency magnetic fields, long-lasting exposure.

Introduction

Current literature data indicate that exposure to extremely low frequency (ELF) magnetic fields may influence intensification of redox reactions and activity of enzymes decomposing oxygen free radicals in cells, which results in subsequent changes in oxygen free radicals levels in living tissues [1, 2, 3, 4]. According to one of the hypotheses [5] the process of aging could be related to cumulation of a prolonged action of oxygen free radicals on tissues and on progressive decrease in antioxidant activity of the organism. Since the inhibition of estrogen activity during menopause develops acceleration of aging processes in many tissues (potentially related to stimulation of free radicals action) [5] the aim of our experiment was to estimate the effect of long-lasting exposure to ELF magnetic fields on some oxygen free radicals activity indicators content in hepatic tissue homogenats in previously experimentally ovariectomised female rats.

Materials and methods

The experiment was carried out on 35 female Wistar rats (age: 10 month, weight: 300 ± 50 g). The animals were housed under optimal environmental conditions (constant temperature and humidity of air) in a reverse 12:12 h light-dark cycle. They were fed with standard laboratory food and had free access to water.

The source of an ELF magnetic field was an Ambit 2000 (Poland) unit consisting of variable magnetic field generator producing magnetic fields of different physical parameters, and of a cylindrical applicator which enables whole animal body exposure.

The animals were randomly divided into 4 groups.

I group „5" consisted of 10 rats ovariectomised in the 6^{th} month of age and subsequently exposed to a rectangular magnetic field at a frequency of 5 Hz and magnetic flux density of 10 mT.

II group „40" consisted of 9 rats ovariectomized in the 6th month of age and subsequently exposed to a sinusoidal magnetic field at a frequency of 40 Hz and magnetic flux density of 10 mT.

III group „O" consisted of 8 rats ovariectomized in the 6th month of age and subsequently exposed to a sham-exposure.

IV control group consisted of 8 non-ovariectomised rats exposed to a sham-exposure.

In all groups, the whole body exposure lasted 1 hour daily for 4 month.

After the end of the exposure cycle animals were exsanguinated in ether narcosis. Segments of hepar were obtained to prepare 10% tissue homogenats in a teflon/glass system subsequently frozen. After defreezing the samples were desintegrated with the use of ultrasound. Lowry's method was used to establish protein levels in samples examined. In order to estimate the effect of a magnetic field on changes in oxygen free radicals in tissues the amount of some indirect indicators of free radicals activity such as antioxidant enzymes: glutathione peroxidase (POX), CuZn-stimulated superoxide dismutase (CuZnSOD) and glutathione-S-transferase (GST) and amount of malon dialdehyde (MDA) were determinated in 1 g samples according to [6, 7, 8].

The results from each group were statistically analysed with a STATISTICA programme using the Kruskal-Wallis rang ANOVA test and the post-hoc U Mann-Whitney test.

Results

The amounts of antioxidant enzymes POX, CuZnSOD and GST in hepatic tissue homogenats in experimental group of ovariectomised female rats did not differ significantly compared to the control group of non-ovariectomised female rats (Figs. 1-3).

Decrease in the tissue content of antioxidant enzymes was observed in rats from both groups exposed to a magnetic field compared to rats from the group in which a sham-exposure was made, but the differences were significant only for POX (group „5" — $p = 0.001$, group „40" — $p = 0.012$) (Fig. 3).

The amount of MDA in hepatic tissue homogenats of rats from the ovariectomised sham-exposed group was insignificantly higher ($p = 0.14$) compared to the group of non-ovariectomised rats (Fig. 4). The exposure to a magnetic field caused a significant

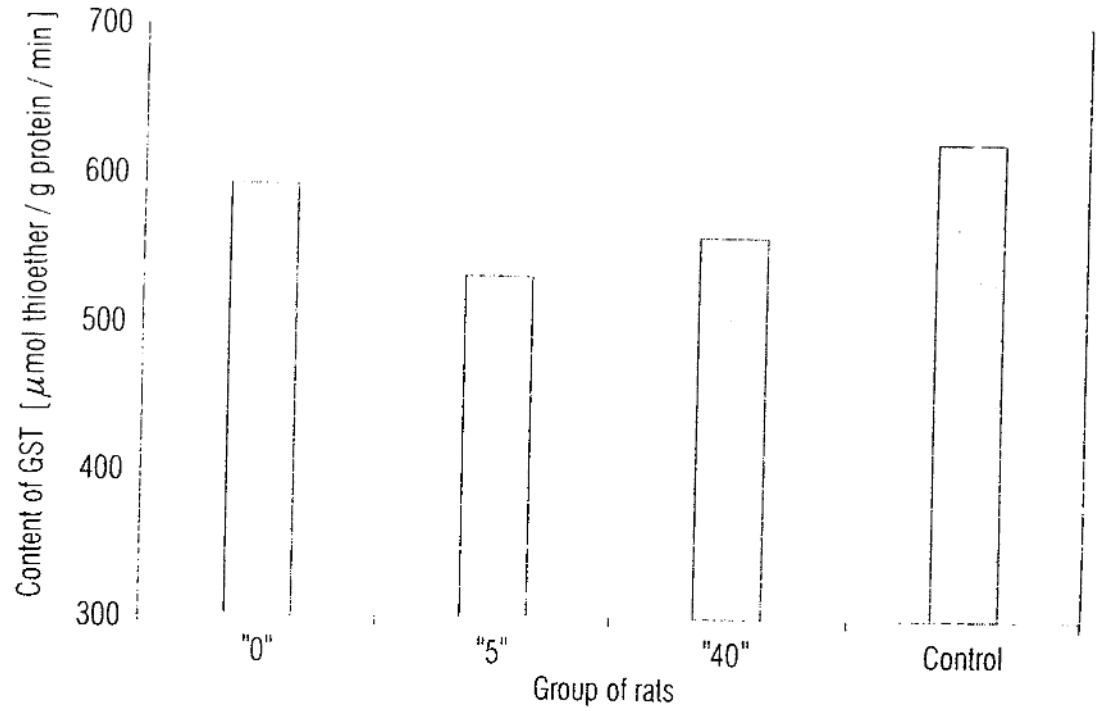

Figure 1. The content of glutathione-S-transferase (GST) in hepatic tissue homogenats in the control group of non-ovariectomised female rats and in both groups: „5" and „40" of ovariectomised female rats exposed to a magnetic field of different parameters as compared to the sham-exposed group „0", with statistical evaluation

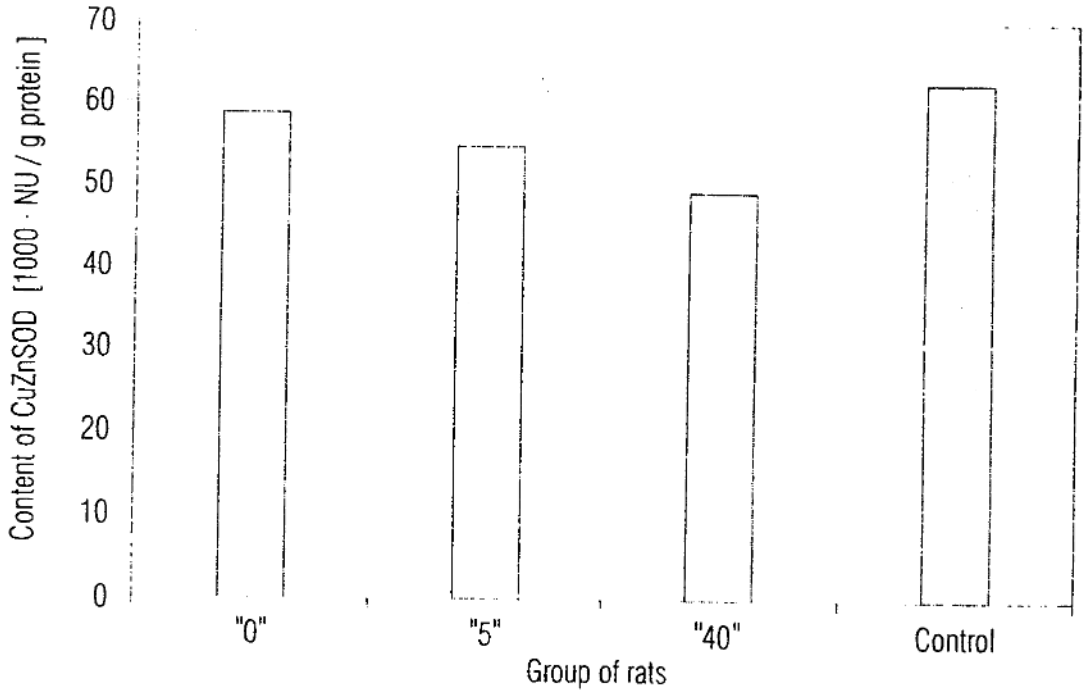

Figure 2. The content of CuZn-stimulated dismutase (CuZnSOD) in hepatic tissue homogenats in the control group of non-ovariectomised female rats and in both groups: „5" and „40" of ovariectomised female rats exposed to a magnetic field of different parameters as compared to the sham-exposed group „0", with statistical evaluation

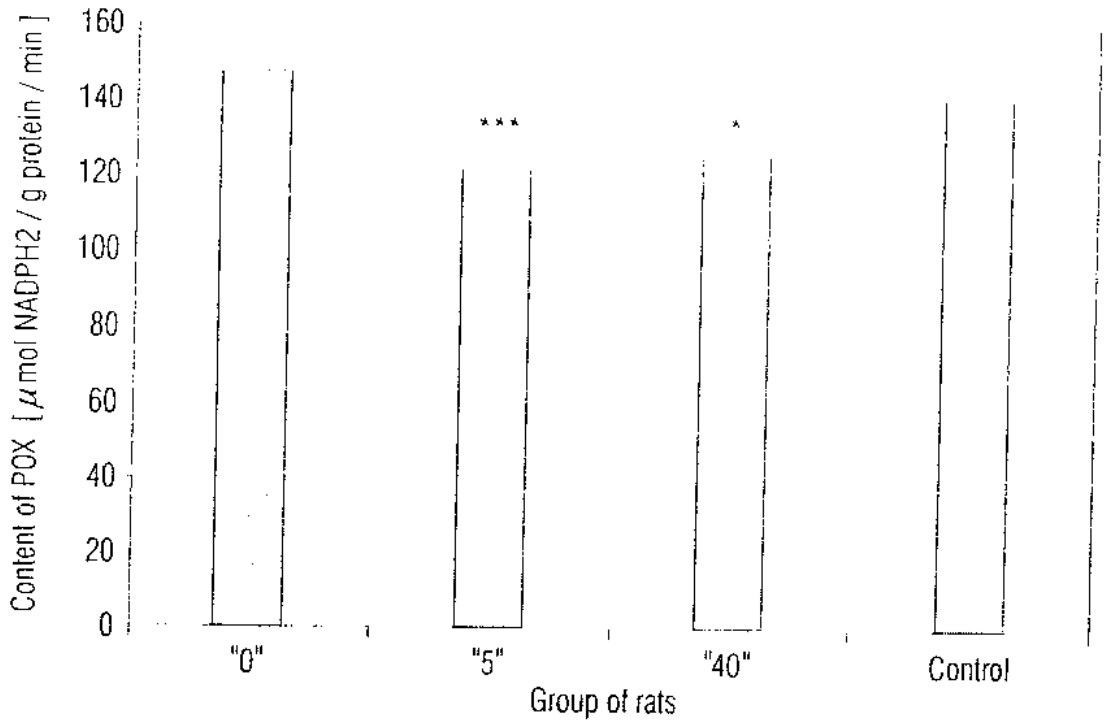

Figure 3. The content of glutathione peroxidase POX in hepatic tissue homogenats in the control group of non-ovariectomised female rats and in both groups: „5" and „40" of ovariectomised female rats exposed to a magnetic field of different parameters as compared to the sham-exposed group „0", with statistical evaluation ($^{*}p < 0.05$, $^{***}p < 0.001$)

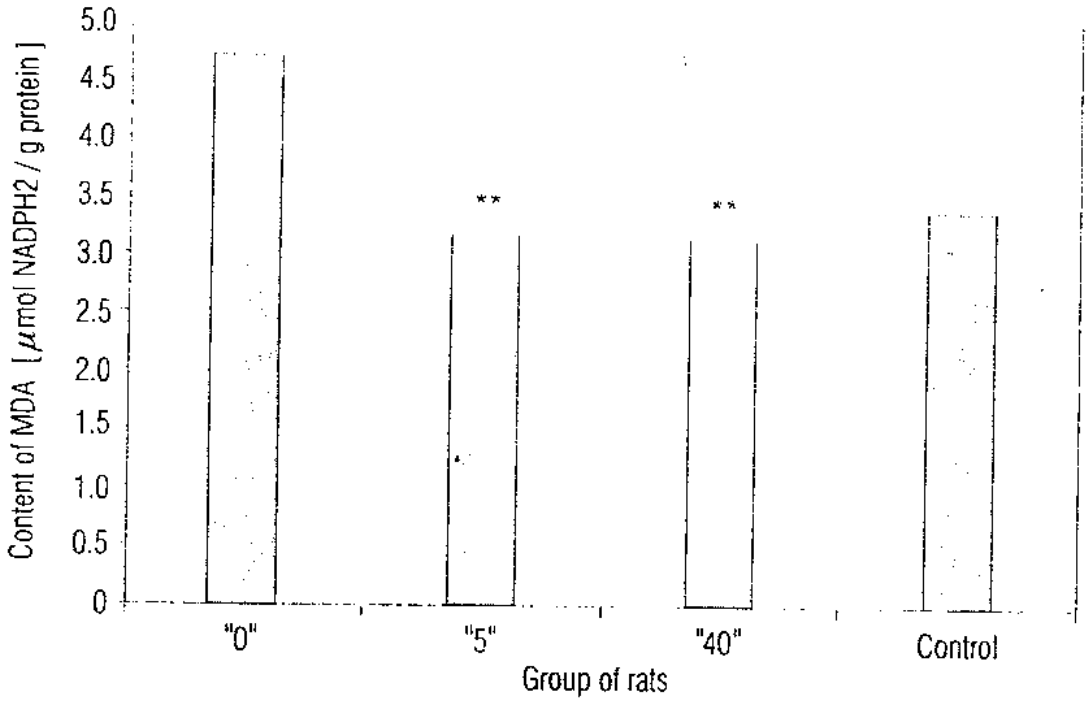

Figure 4. The content of dimalonic aldehyde (MDA) in hepatic tissue homogenats in the control group of non-ovariectomised female rats and in both groups: „5" and „40" of ovariectomised female rats exposed to a magnetic field of different parameters as compared to the sham-exposed group „0", with statistical evaluation ($^{**}p < 0.01$)

decrease in MDA tissue content in both groups of rats exposed to an ELF magnetic field (group „5" — $p = 0.003$, group „40" — $p = 0.007$). The results in both groups of exposed rats were comparable to those of non-ovariectomised rats (Fig. 4).

Discussion

Oxygen free radicals produced in many enzymatic reactions affect indirectly transformations of nuclear proteins by oxidation of membrane amin-acid radicals, which results in inactivation of enzymes and increase in the susceptibility to proteolysis [3, 4, 9]. It has also been observed that free radicals effect is related to stimulation of calcium ions dislocation on ion channels and cytoplasm level, and to stimulation of protein phosphorylation processes and activation of nuclear transcription factors with subsequent acceleration of growth or especially death of cells [9]. The increase in tissue content of oxygen free radicals usually leads to an unfavourable biological process ending in cell apoptosis [10, 11].

Peroxidase, dismutase and glutathione transferase are antioxidant enzymes which protect the organism against oxygen free radicals action. The increase in antioxidant enzymes activity indicates the induction of their synthesis and indirectly confirms the stimulation of free radicals production. The decrease in tissue content of these enzymes is caused by either lower requirement or inhibition of their synthesis. Malon dialdehyde is a product of long lasting free radicals action on cellular lipids, and increase in its tissue content indicates high activity of free radicals in cells.

It has been proved in many experiments [4, 11, 12, 13] that ELF magnetic fields affect the activity of enzymatic reactions, stimulate protein synthesis and intensity of oxidative-reductive processes in living organisms, which may result in potential influence of the magnetic field on activity of antioxidant enzymes desintegrating oxygen free radicals with subsequent changes in tissue content of these radicals and products of their biological action.

The results of our study did not confirm any significant and harmful influence of long lasting exposure to a magnetic field on antioxidant enzymes content in hepatic tissue homogenats and their effective protecting action in ovariectomised female rats, since the only significant decrease in POX tissue content observed was probably due to lower requirement for this enzyme. On the other hand, the significant decrease in the previously increased MDA tissue content in ovariectomised female rats also points to

favourable influence of a variable magnetic field on oxygen free radicals activity in living organisms during menopause. On the basis of the results obtained we may conclude that an ELF magnetic field indirectly inhibits the action of oxygen free radicals.

The final and unequivocal assessment of the observed effect needs further investigation with use of various experimental models.

Conclusion

Long lasting exposure to an ELF magnetic field does not affect antioxidant enzymes activity in hepatic tissue of ovariectomised rats and has a favourable effect on oxygen free radicals activity, which results in a significant decrease in the previously increased malon dialdehyde tissue content.

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