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EFFECT OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS ON CELL PROLIFERATION, ANTIOXIDATIVE ENZYME ACTIVITIES AND LIPID PEROXIDATION IN 3T3-L1 PREADIPOCYTES - AN *IN VITRO* STUDY

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The exposure to extremely low frequency electromagnetic field (ELF-MF, frequencies less than 200-300 Hz) can alter the transcription and translation of genes, influence the cell proliferation rate and affect enzyme activities. Moreover, the hypothesis that ELF-MF increases free oxygen metabolites generation has been proposed. Since recent *in vivo* studies suggest that electric and magnetic fields are able to affect adipose cells metabolism. The aim of the study was to examine the effects of ELF-MF (frequency of basic impulse 180-195 Hz, induction 120 μT) on cell proliferation, antioxidative enzyme activities and malondialdehyde (MDA) concentration in 3T3-L1 preadipocyte cell culture. We found that ELF-MF application lasting 36 minutes daily failed to influence cell count after 24h and 48 h of incubation. After 24 h, in the ELF-MF treated group, manganese- and copper-zinc-containing superoxide dismutase (MnSOD and Cu/ZnSOD) isoenzymes media activities were decreased, catalase activity was increased, whereas there were no significant differences in glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-Rd) activities in comparison to the control. After 48 h of incubation, all enzyme activities were reduced, except for GSSG-Rd, in which no changes were noticed. MDA concentration at 24 h after incubation with the exposure to ELF-MF was significantly higher in comparison to the control, without ELF-MF. After 48 h of incubation, MDA levels were significantly lower in both groups with no differences between the groups without and with ELF-MF. We conclude that ELF-MF influences antioxidative enzyme activities and increases lipid peroxidation in 3T3-L1 preadipocyte cultures.

Key words: *3T3-L1 preadipocytes, extremely low frequency electromagnetic field, antioxidative enzyme activities, malondialdehyde*

INTRODUCTION

Adipose tissue metabolism is regulated by humoral and neuronal mechanisms. Among hormonal factors, there are many hormones, such as leptin (1), cholecystokinin (2), ghrelin (3), secretin (4) and melatonin (5) which, acting directly or indirectly, influence adipose tissue. Many of these hormones play an important role in the brain-gut axis (6, 7).

Recent *in vivo* studies suggest that physical factors, including 10 kV, 30 μT, 60 Hz electric and magnetic fields, are able to affect adipose tissue metabolism (8). There is an agreement that exposure to an extremely low frequency electromagnetic field (ELF-MF, frequencies less than 200-300 Hz) can alter the transcription and translation of genes, such as *hsp70, myc* and *jun* (9) and leads to generation of free oxygen radicals (10). There are different effects, from inhibition to stimulation of cell proliferation, after exposure to ELF-MF, which depends on the signal parameters of amplitude and frequency of the magnetic field (11). Exposure to 50 and 60 Hz electromagnetic fields causes an increase in acetylcholinesterase and alkaline phosphatase activities in early chicken embryos (12). In contrast, exposure to a 30 Hz magnetic field evokes a progressive inhibition of alkaline phosphatase activity (13).

Recent studies demonstrate that ELF-MF influences the synthesis and release of melatonin, and, in this way, could influence oxidative/antioxidative status. Our previous *in vitro* experiments demonstrated that melatonin affects 3T3-L1 preadipocytes, which results in stimulation of cell proliferation, an increase in antioxidative enzyme activities and a decrease in lipid peroxidation (14). On the other hand, Reiter *et al* revealed that exposure to an electromagnetic field at night depresses a conversion of serotonin to melatonin within the pineal gland and severely attenuates the nighttime rise in pineal melatonin production (15). In contrast, *in vivo* studies have shown that acute exposure to a 50 Hz magnetic field has no effect on nighttime secretion of melatonin in humans (16, 17).

Our previous study demonstrated that ELF-MF attenuates antioxidative action of melatonin in AT478 murine squamous cell carcinoma culture (18). The aim of the present study was to examine effects of ELF-MF on cell proliferation and antioxidative enzyme status in 3T3-L1 - preadipocyte cell culture.

MATERIAL AND METHODS

Cell culture

3T3-L1 cells are preadipocytes obtained by Green and Kehinde from murine 3T3 fibroblasts by cloning clusters of cells filled with fat droplets (19). 3T3-L1 cells were purchased from ATCC (American Type Culture Collection, Rockville, MD, USA). Preadipocytes were plated at a density of 1 x 10^6 cells per 25 cm^2 flask and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin under an atmosphere of 95% air and 5% CO_2 at 37°C.

Experimental protocol

To study the influence of ELF-MF on 3T3-L1 cells, we used a weak variable magnetic field of saw-like shape of impulse, at a frequency of basic impulse 180-195 Hz and induction 120 μT, generated by the device for magnetostimulation VIOFOR JPS (Med & Life, Komorów, Poland). Cell culture dishes were exposed to magnetic fields for a period of 36 minutes between 13.00 and 16.00 for two days.

3T3-L1 cell media were not changed during the observation time (up to 48 h). After incubation periods, media were removed, centrifuged and freezed until enzymatic measurements. Cells were trypsinized and the number of cells was estimated by direct counting, using a net micrometer with 10x objective and 10x ocular, on three square (1 mm^2). We present the cell number (cells/ml) as average count per square x 10000. Viability of cells was estimated by trypan blue staining. The cell monolayer was discarded, harvested and treated with trypan blue.

Enzymatic assays

Manganese-containing (MnSOD) and copper-zinc-containing superoxide (Cu/ZnSOD) dismutase isoenzyme (EC 1.15.1.1) activities were estimated according to Oyanagui (20) and expressed in nitrite units/ml (NU/ml). Glutathione peroxidase (GSH-Px) (EC 1.11.1.19) activity was measured according to Paglia and Valentine using enzymatic conjunction with glutathione reductase (21, 22). Catalase (CAT) (EC 1.11.1.6) activity was measured according to the kinetic method of Aebi (23) and expressed in IU/ml of medium. Glutathione reductase (GSSH-Rd) (EC 1.6.4.2) assay was based on the oxidation of NADPH to $NADP^+$ (24).

MDA concentrations were determined according to the colorimetric method by Ohkawa *et al* using the reaction with thiobarbituric acid (25).

Drugs

The following agents were used: trypsin, trypan blue, Penicillin-Streptomycin Mixture, Dulbecco's Modified Eagle's Medium and Fetal Bovine Serum (Bio Whittaker, Verviers, Belgium).

Statistics

All values are given as means ± SEM. Comparison of differences between the mean values were made using ANOVA and Student's t-test. Differences with $P < 0.05$ were considered as statistically significant.

RESULTS

Influence of ELF-MF on 3T3-L1 cells proliferation

ELF-MF application at a frequency of basic impulse 180-195 Hz and induction 120 μT lasting 36 minutes daily did not influence cell count after 24 and 48 h of incubation in comparison to the control (data not shown).

Influence of ELF-MF on antioxidative enzyme activities in 3T3-L1 cells media

After 24 h of incubation and twice ELF-MF application MnSOD and Cu/ZnSOD activities were significantly decreased while CAT activity was

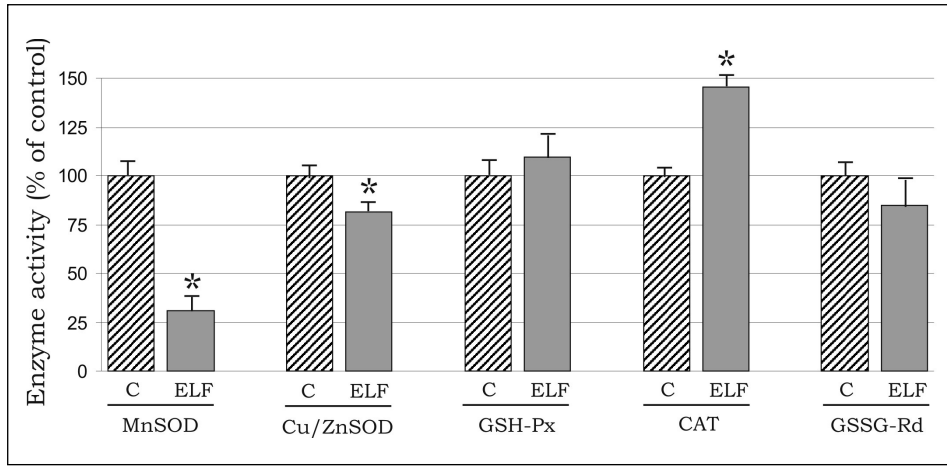

Fig. 1. Antioxidative enzyme activities in 3T3 L1 cell culture media after 24 h of incubation. Data are given as percentages of the initial value (mean ± SEM); initial activities of MnSOD, Cu/ZnSOD, CAT, GSH-Px and GSSG-Rd in the control group are 2.34 ± 0.11 NU/ml, 3.11 ± 0.09 NU/ml, 84.11 ± 6.11 IU/ml, 328.7 ± 21.1 μmol $NADPH_2$/ml and 2.21 ± 0.11 IU/ml, respectively. * P < 0.05 *vs.* the control group.

significantly increased and there were no significant differences in GSH-Px and GSSG-Rd activities in comparison to the control values (*Fig. 1*).

After 48 h of incubation, all media enzyme activities were reduced except for GSSG-Rd, where no changes were noticed (*Fig. 2*).

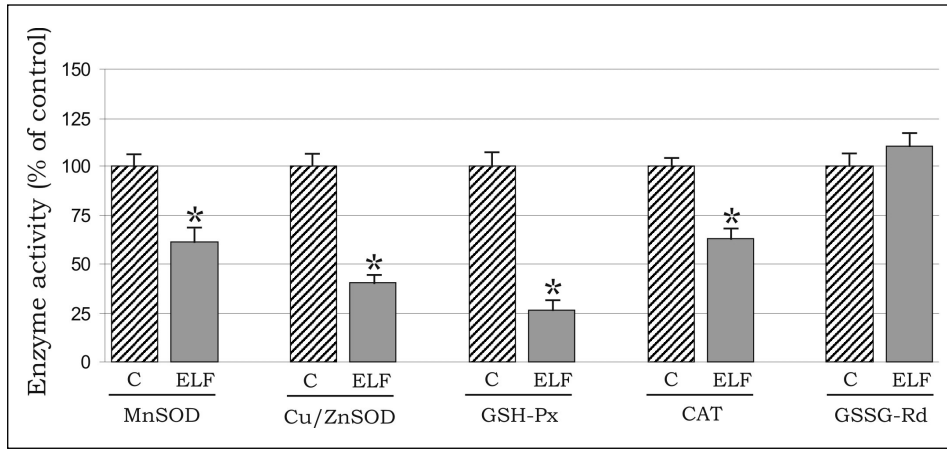

Fig. 2. Antioxidative enzyme activities in 3T3 L1 cell culture media after 48 h of incubation. Data are given as percentages of the initial value (mean ± SEM); initial activities of MnSOD, Cu/ZnSOD, CAT, GSH-Px and GSSG-Rd in the control group are 1.21 ± 0.07 NU/ml, 3.36 ± 0.11 NU/ml, 162.7 ± 10.32 IU/ml, 132.7 ± 11.1 μmol $NADPH_2$/ml and 1.09 ± 0.07 IU/, respectively. *P < 0.05 *vs.* the control group.

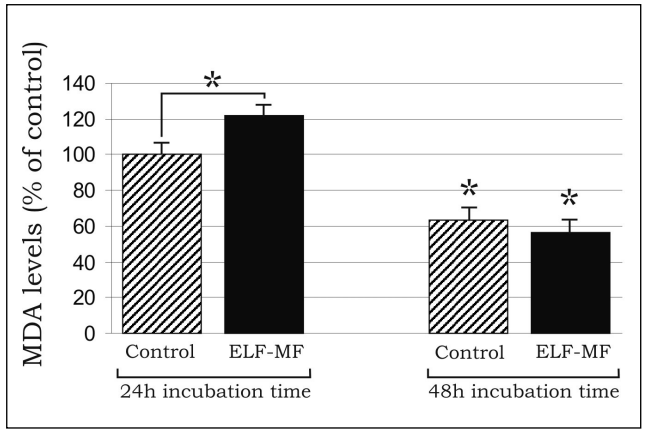

Fig. 3. Concentrations of MDA in 3T3 L1 cell culture media after 24 and 48 h of incubation. Data are given as percentages of the initial value (mean ± SEM); initial concentration of MDA in the control group is 2.55 ± 0.21 μmol/ml. * $P < 0.05$ *vs.* MDA concentration in the control group after 24 h of incubation.

Influence of ELF-MF on MDA concentration in 3T3-L1 cells media

MDA media concentration 24 h after incubation and treatment with ELF-MF was significantly higher in comparison to the control (*Fig. 3*). In contrast, after 48 h of incubation, MDA levels were lower in both groups, compared to the control concentration after 24 h of incubation. There were no differences between the two groups in MDA level 48 h after incubation (*Fig. 3*).

DISCUSSION

Results of the present *in vitro* study demonstrate for the first time that ELF-MF is able to affect activities of antioxidative enzymes and lipid peroxidation in 3T3-L1 preadipocyte cultures.

The cell line used offers an excellent model to study the differentiation process, during which 3T3-L1 cells undergo a change from their elongated fibroblastic shape to an oval form and accumulate small drops of lipids within the cytoplasm (19). These lipid drops fuse into one large drop giving the cell the aspect of a mature adipocyte of white adipose tissue (19).

Our study demonstrates that ELF-MF at a frequency of basic impulse of 180-195 Hz and induction equal to 120 μT, generated by the device for magnetostimulation, failed to influence 3T3-L1 cells proliferation. Interestingly, Pirozzoli *et al.* demonstrate an increase in the proliferation index in neuroblastoma cell line LAN-5 after 7 days of continuous exposure to a 50 Hz magnetic field (26). In contrast, Hoffmann *et al.* show a reduction of granule cells proliferation rates after exposure to 50 Hz electromagnetic field (27).

Experimental data demonstrate that exposure to ELF-MF leads to generation of reactive oxygen species (ROS) (9). On the other hand, ROS are known to alter antioxidant enzyme activities. It has been proposed that moderate levels of ROS can induce an increase in antioxidant enzyme activities, whereas very high level

of these reactants was shown to attenuate antioxidant enzyme activities (28). ROS are generated *in vivo* conditions in extremely high amounts during partial hypoxia and subsequent reoxygenation, for example in haemorrhagic shock and reperfusion (29, 30), or when the organ is exposed to toxic environmental agents, for example ionising radiation (31).

Free radicals have been implicated in the biological effects of ELF-MF since an exposure of human neutrophils to a 22 mT, 60 Hz magnetic field resulted in a significant increase of both, superoxide anion production (by 26.5%) and beta-glucuronidase release (by 53%) (32). Since ROS are able to damage many biomolecules, including DNA, enzymes, lipids and proteins, we can hypothesize that a reduction in antioxidative enzyme activities observed in our study can be related to overproduction of ROS under ELF-MF exposure.

Results of the present study demonstrate, for the first time, that ELF-MF is able to influence oxidative-antioxidative balance in 3T3-L1 preadipocytes; however, the precise mechanisms involved in this effect are not clear. We found a decrease in activities of MnSOD and Cu/ZnSOD and an increased activity of CAT after 24 h of cell incubation with ELF-MF application. This can be attributed to the enhancement in the generation of ROS and induction of the activity of CAT which metabolises H_2O_2 to water. On the other hand, the increased amounts of ROS could also be explanatory for a reduction in MnSOD and Cu/ZnSOD activities after 24 h of incubation.

Our results show a decrease in activities of all enzymes studied, except for GSSG-Rd activity, after 48 h of incubation with or without ELF-MF application. This effect can be explained by the action of ROS, generated in large amounts upon ELF-MF application. Interestingly, the action of ELF-MF depends on magnetic field parameters and the type of cell studied. In our previous report we have demonstrated that application of ELF-MF (frequency 3 Hz to 3kHz, induction >1 mT) on AT478 murine squamous cell carcinoma culture caused an increase of both, MnSOD and CU/ZnSOD isoenzyme activities (18). Interestingly, ELF-MF decreases antioxidative enzyme activities in AT478 murine squamous cell carcinoma culture containing melatonin in the cell medium (18).

Free radicals-induced cell damage may be quantitatively determined by measurement of MDA level, which is an indicator of lipid peroxidation. Our present results demonstrate an increased level of MDA in the media of cells treated with ELF-MF after 24 h of incubation. Conversely, there are no differences between the two groups in MDA concentrations after 48 h of incubation. The former results are difficult to explain, especially so as activities of almost all studied antioxidative enzymes are decreased after 48 h of incubation. Similarly, we have reported an increased MDA concentrations and Cu/ZnSOD and MnSOD activities in AT478 murine squamous cell carcinoma culture (18).

In conclusion, our results suggest that ELF-MF application influences antioxidative enzyme activities and lipid peroxidation in 3T3-L1 preadipocytes media without affecting proliferation rate of 3T3-L1 cells.

REFERENCES

1. Shimizu H, Shimomura Y, Hayashi R, et al. Serum leptin concentration is associated with total body fat mass, but not abdominal fat distribution. *Int J Obes Relat Metab Disard* 1997; 21: 536-541.
2. Rehfeld JF, Bundgaard JR, Friis-Hansen L, Goetze JP. On the tissue specific processing of procholecystokinin in the brain and gut - a short review. *J Physiol Pharmacol* 2003; 54 (Suppl 4): 73-79.
3. Peeters TL. Central and peripheral mechanisms by which ghrelin regulates gut motility. *J Physiol Pharmacol* 2003; 54 (Suppl 4): 95-103.
4. Chey WY, Chang T. Neural control of the release and action of secretin. *J Physiol Pharmacol* 2003; 54 (Suppl 4): 105-112.
5. Reiter RJ, Tan D, Mayo JC, Sainz RM, Leon J, Bandyopadhyay D. Neurally-mediated and neurally-independent beneficial actions of melatonin in the gastrointestinal tract. *J Physiol Pharmacol* 2003; 54 (Suppl 4): 113-125.
6. Konturek SJ, Pawlik WW, Dajani EZ. Brain-gut axis in the gastrointestinal system: introductory remarks. *J Physiol Pharmacol* 2003; 54 (Suppl 4): 3-7.
7. Konturek SJ, Konturek PC, Pawlik T, Brzozowski T. Brain-gut axis and its role in the control of food intake. *J Physiol Pharmacol* 2004; 55: 137-154.
8. Burchard JF, Monardes H, Nguyen DH. Effect of 10 kV, 30 microT, 60 Hz electric and magnetic fields on milk production and feed intake in nonpregnant dairy cattle. *Bioelectromagnetics* 2003; 24: 557-563.
9. Chen G, Upham BL, Sun W, et al. Effect of electromagnetic field exposure on chemically induced differentiation of friend erythroleukemia cells. *Environ Health Perspect* 2000; 108: 967-972.
10. Jajte J, Zmyslony M. The role of melatonin in the molecular mechanism of weak, static and extremely low frequency (50 Hz) magnetic fields (ELF). *Med Pr* 2000; 51: 51-57.
11. Ross SM. Combinated DC and ELF magnetic fields can alter cell proliferation. *Bioelectromagnetics* 1990; 11: 27-36.
12. Moses GC, Martin AH. Effects of extremely low-frequency electromagnetic fields on three plasma membrane-associated enzymes in early chicken embryos. *Biochem Int* 1992; 28:659-64.
13. McLeod KJ, Collazo L. Suppression of a differentiation response in MC-3T3-E1 osteoblast-like cells by sustained, low-level, 30 Hz magnetic field exposure. *Radiat Res* 2000; 153: 706-714.
14. Zwirska-Korczala K, Jochem J, Adamczyk-Sowa M, et al. Influence of melatonin on cell proliferation, antioixidative enzyme activities and lipid peroxidation in 3T3-L1 preadipocytes - in vitro study. *J Physiol Pharmacol* (in press).
15. Reiter RJ. Static and extremely low frequency electromagnetic field exposure: reported effects on the circadian production of melatonin. *J Cell Biochem* 1993; 51: 394-403.
16. Crasson M, Becers V, Pequeux C, Claustrat B, Legros JJ. Daytime 50 Hz magnetic field exposure and plasma melatonin and urinary 6-sulfatoxymelatonin concentration profiles in humans. *J Pineal Res* 2001; 31: 234-241.
17. Kurokawa Y, Nitta H, Imai H, Kabuto M. Acute exposure to 50 Hz magnetic fields with harmonics and transient components: lack of effects on nighttime hormonal secretion in men. *Bioelectromagnetics* 2003; 24: 12-20.
18. Zwirska-Korczala K, Adamczyk-Sowa M, Polaniak R, et al. Influence of extremely-low-frequency magnetic field on antioxidative melatonin properties in AT478 murine squamous cell carcinoma culture. *Biol Trace Elem Res* 2004; 102: 227-243.
19. Alvarez M. 3T3 cells in adipocytic conversion. *Arch Invest Med (Mex)* 1991; 22: 235-241.
20. Oyanagui Y. Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal Biochem* 1984; 142: 290-296.

21. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med* 1967; 70: 158-169.
22. Zwirska-Korczala K, Jochem J, Rybus-Kalinowska B, Polaniak R, Birkner E: Assessment of blood superoxide dismutase and glutathione peroxidase activities, and malondialdehyde concentration as oxidation status parameters in obese women. *Pol Arch Med Wewn* 2003; 110: 725-731.
23. Aebi H. Catalase in vitro. *Methods Enzymol* 1984; 105: 121-126.
24. Carlberg I, Mannervik B. Glutathione reductase. *Methods Enzymol* 1985; 113: 484-490.
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979; 95: 351-358.
26. Pirozzoli MC, Marino C, Lovisolo GA, Laconi C, Mosiello L, Negroni A. Effects of 50 Hz electromagnetic field exposure on apoptosis and differentiation in a neuroblastoma cell line. *Bioelectromagnetics* 2003; 24: 510-516.
27. Hoffmann K, Bagorda F, Stevenson AF, Teuchert-Noodt G. Electromagnetic exposure effects the hippocampal dentate cell proliferation in gerbils (Meriones unguiculatus). *Indian J Exp Biol* 2001; 39: 1220-1226.
28. Brydon L, Petit L, Delagrange P, Strosberg AD, Jockers R. Functional expression of MT2 (Mel1b) melatonin receptors in human PAZ6 adipocytes. *Endocrinology* 2000; 142: 4264-4271.
29. Jochem J. Involvement of the renin-angiotensin system in endogenous central histamine-induced reversal of critical haemorrhagic hypotension in rats. *J Physiol Pharmacol* 2004; 55: 39-55.
30. Jochem J, Zwirska-Korczala K, Gwozdz B, Walichiewicz P, Josko J. Cardiac and regional haemodynamic effects of endothelin-1 in rats subjected to critical haemorrhagic hypotension. *J Physiol Pharmacol* 2003; 54: 383-396.
31. Karbownik M, Reiter RJ. Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. *Proc Soc Expl Biol Med* 2000; 225: 9-22.
32. Khadir R, Morgan JL, Murray JJ. Effects of 60 Hz magnetic field exposure on polymorphonuclear leukocyte activation. *Biochim Biophys Acta* 1999; 1472: 359-367.

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