Cent. Eur. J. Biol.
DOI: 10.2478/s11535-014-0335-9

VERSITA

Central European Journal of **Biology**

Effect of ELF-EMF on antioxidant status and micronuclei in K562 cells and normal lymphocytes

Research Article

Branislava Srdjenovic[1]*, Jasminka Mrdjanovic[2], Aleksandra Jovanovic Galovic[3], Nebojsa Kladar[1], Biljana Bozin[1], Vladimir Jurisic[4], Gordana Bogdanovic[2]

[1]*Department of Pharmacy, Faculty of Medicine, University of Novi Sad, 21000 Novi Sad, Serbia*

[2]*Department of Experimental Oncology, Oncology Institute of Vojvodina, 21208 Sremska Kamenica, Serbia*

[3]*Virusology Center, Public Health Institute of Vojvodina, 21000 Novi Sad, Serbia*

[4]*Faculty of Medicine, University of Kragujevac, 34000 Kragujevac, Serbia*

Received 27 February 2014; Accepted 25 April 2014

Abstract: The effect of ELF-EMF on DNA through changes in antioxidative enzyme activities has not been sufficiently explored yet. The aim of this study was to determine ELF-EMF effect on antioxidative enzymes in cancer cell line and genotoxic potential on normal human lymphocytes. K562 cells were exposed to 50 Hz ELF-EMF (40 μT, 100 μT; 3 h, 24 h) and spectrophotometric determination of lipid peroxidation and antioxidative enzyme activities was conducted. Genotoxicity of ELF-EMF (50 Hz, 100 μT) was investigated by cytokinesis-block micronucleus assay in a normal human lymphocytes (exposure 24 h and 48 h). Results demonstrated that ELF-EMF did not alter the process of lipid peroxidation and superoxide dismutase activity. Catalase activity was increased only after application of 100 μT EMF for 24 h. Glutathione-S-transferase and -reductase activities were increased. Treatment with 100 μT ELF-EMF (24 h, 48 h) significantly reduced micronuclei incidence, while cell proliferation was significantly increased. Results indicate that 50 Hz ELF-EMF (40 μT, 100 μT) are week stressors which alone cannot generate enough ROS to induce process of lipid peroxidation in cancer cell line but strong enough to induce response of antioxidative system. Furthermore, 100 μT ELF-EMF in human lymphocytes did not exhibit genotoxic potential during 24 h and 48 h treatment, but stimulated cell proliferation.

Keywords: *ELF-EMF • Human leukemia cell line • Oxidative stress • Lymphocytes • Micronuclei • Genotoxicity*

1. Introduction

Electromagnetic fields (EMF) of all frequencies represent one of the most common and fastest growing environmental influences, about which anxiety and speculations are spreading. All populations are now exposed to varying degrees of EMF, and the levels will continue to increase as technology advances. There is no doubt that short-term exposure to very high levels of electromagnetic fields can be harmful to health. Current public concern focuses on possible long-term health effects caused by exposure to electromagnetic fields at levels below those required to trigger acute biological response [1]. The potential effects of extremely low frequency electromagnetic fields (ELF-EMF) on human health is a matter of public concern, due to the increasing use of electric power for domestic and industrial appliances in the last few years. ELF fields generally have frequencies up to 300 Hz. In the IARC (International Agency for Research on Cancer) evaluation, ELF magnetic fields were classified into group "2B" ("possibly carcinogenic to humans").

* *E-mail: srdjbr@yahoo.com*

Limited evidence of carcinogenicity in humans was chiefly based on epidemiological studies showing a consistent association between magnetic fields above 0.3/0.4 μT and the risk of childhood leukemia. For cancers other than childhood leukemia there was either inadequate evidence or some evidence against an association [2].

Different *in vivo* and *in vitro* studies have shown a possible co-promoter influence of ELF-EMF on various biological functions and tumor growth [3]. However, the molecular mechanism through which ELF-EMF can influence cellular behavior is still unclear. A possible hypothesis is that ELF-EMF can interfere with chemical reactions involving free radical production [4] and in the last few years, some data from literature have described redox-related cellular changes, following ELF-EMF exposure [5,6]. Free radicals are known to affect various biochemical pathways and reactions including cell proliferation, cellular differentiation and apoptosis. Previous studies showed that ELF-EMF increased the levels of reactive oxygen species [6]. Resistance to cell death – particularly apoptotic cell death – is an important aspect of tumorigenesis. Many tumor cells appear to have constitutively elevated levels of heat-shock proteins (HSP), which serve to protect them against apoptosis, thus causing chemotherapeutic resistance and increased tumorigenesis [2]. Exposure to ELF-EMF has been linked to increased incidence of leukemia and other tumors and increase of HSP by ELF-EMF may be correlated to these results. On the other hand, increase in free radicals are known to induce apoptosis and ELF-EMF has been shown to increase free radical levels [7]. The effect of ELF-EMF on biological systems must be considered to be dependent on the status of the system. The results of Garip and Akan demonstrate that ELF-EMF decreases the number of apoptotic cells of an erythroleukemia cell line by increasing heat-shock protein levels albeit an increase in reactive oxygen species (ROS). However, as a co-stressor, it augments the effect of oxidative stress on the number of apoptotic cells, one reason being the stabilizing effect on free radicals. The increase in heat-shock protein levels induced by ELF-EMF is not able to reverse the increase in the number of apoptotic cells caused by oxidative stress [7]. Based on the results of Ayse and co-workers about influence of ELF-EMF on differentiation of K562 cancer cell line, it can be concluded that ELF-EMF exposure at different time-courses results in different outcomes [8].

Regarding genotoxicity of ELF-EMF, although there is no consistent data about the degree of risk, it is assumed that the ELF-EMF do not act directly on DNA but it can affect the increase of the free radicals in cells and thus indirectly promote oxidative radical attack on DNA [9]. It is, therefore, important to investigate the antioxidative (AO) enzyme activity as non-genotoxic element, along with a biomarker of genotoxicity, which might contribute to the elucidation of activities at the cellular level influenced by ELF-EMF.

Micronuclei test, indicative for DNA damage, is one of the most widely used assays for investigation of genotoxicity. Micronucleus formation can either result from acentric fragments derived from DNA double strand breaks or chromosomal non-disjunction due to damage to kinetochore proteins. Recent genotoxicity investigations of ELF-magnetic demonstrated inconsistent results with 22% studies positive, 46% negative, and 32% inconclusive [10]. The epidemiological association between ELF-EMF and leukemia should be emphasized, since it indicates that lymphocytes are the target cells for electromagnetic radiation [2].

Therefore our goal was to investigate the effect of ELF-EMF on antioxidative status in leukemia cell lines as well as to determine the degree of DNA damage in normal human peripheral blood lymphocytes after ELF-EMF exposition.

Based on the above-mentioned very complex EMF effects on various biological systems and considering that the effects of extremely low frequency radiation depend on the strength of the magnetic field source, distance from the source and the time spent in the magnetic field. The aim of this study was to examine the impact of the strength and the length of exposure to ELF-EMF on antioxidant enzymes K562 cell line and micronuclei formation in normal lymphocytes. In the study we used the EMF frequency of 50 Hz, and magnetic induction of 40 μT and 100 μT, since they are most commonly found in the living and working environment.

2. Experimental Procedure

2.1 Exposure system

According to the SCENIHR recommendations, exposure has to be performed under fully controlled conditions of field exposure (frequency conditions, flux density), temperature and CO_2 [2].

Electromagnetic field was generated by using four serially connected solenoids placed on a wooden frame (240mm x 240mm x 300mm) which had 26, 11, 11 and 26 coils of copper wire, respectively. The system was limited to these dimensions because it was supposed to be held in a chamber at 37°C. Inside this wooden frame a zone with highly homogeneous electromagnetic field (80 x 80 x 80 mm) was created in which cell cultures were placed. The device was powered using a transformer

connected to power distribution network (220 V~, 50 Hz) with an output of alternate current 0 – 1.3 A~ and voltage 0 – 2.3 V. K562 cell cultures were exposed to an electromagnetic field of 50 Hz frequency, magnetic induction was 40 μT and 100 μT and incubation time of 3 h and 24 h. The current parameters (I, U) were measured by universal measurement instrument and the vector of magnetic induction was measured with EFA-300 instrument (NARDA – Safety Test Solution).

2.2 Cell line

Human erythroleukemia K562 cell line was cultured in 25 mL flasks (Costar, USA) in 10 mL of RPMI1640 medium (Sigma, USA) supplemented with 2 mM L^{-1} L-glutamine, 10% FCS (v/v) (Veterinary institute Novi Sad - NIVNS), 100 IU mL^{-1} penicillin (ICN, USA), and 100 μg mL^{-1} streptomycin (ICN, USA) at 37°C in a humidified 5% CO_2 atmosphere.

K562 cell lines were exposed to an electromagnetic field of 50 Hz frequency, and magnetic induction of an electromagnetic field of 40 μT and 100 μT, for 3 h and 24 h. Every exposed group had its own control. Unexposed control K562 cell line groups were kept in similar conditions without field application to eliminate any differences between the exposed and control groups.

All enzyme activities and TBARS levels analyses were performed in cytosolic cell fraction, in supernatant obtained by ultrasonification (Soniprep 150 MSE) (10 minutes at 10 000 rpm at 4°C). All spectrophotometric measurements were carried out in triplicates (Agilent 8453 UV/VIS spectrophotometer with termostated multicell position sample system).

2.3 Culture of normal peripheral blood lymphocytes

Heparinized whole blood was collected by venous puncture from 10 healthy volunteers and used for the peripheral blood lymphocyte cultures in CBMN test. Briefly, 0.5 mL of the whole blood was added to 5 mL of RPMI 1640 cell culture medium (Sigma, USA) supplemented with 2 mM L^{-1} glutamine, 20% of heat-inactivated fetal calf serum (FCS, NIVNS, Serbia) and antibiotics: 100 IU mL^{-1} of penicillin and 100 μg mL^{-1} of streptomycin (ICN, Serbia). Cell cultures were stimulated for division with phytohemagglutinin (PHA-M, Sigma, USA) at a final concentration of 20 μg mL^{-1} and incubated at 37°C for 72 h in 5% CO_2 atmosphere with 95% humidity.

The lymphocyte cultures were exposed to the electromagnetic field of 50 Hz frequency, and magnetic induction of the electromagnetic field of 100 μT, for 24 h and 48 h. Same samples without field application represented untreated group.

2.4 Lipid peroxidation measurement

Lipid peroxidation (LP) was assayed by spectrophotometric method at 535 nm in which the released MDA served as the index of LP [11]. The level of lipid peroxidation was expressed as nmol MDA/10^6 of cells.

2.5 Superoxide dismutase assay

Total (Cu-Zn and Mn) superoxide dismutase (SOD) activity measuring method was based on the xanthine/xanthine oxidase system [12]. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the cytochrome C reduction rate and the result were expressed as U/10^6 of cells.

2.6 Catalase assay

The principle of the assay is based on monitoring the H_2O_2 decomposition rate at 240 nm [12]. Results were expressed as U/10^6 of cells.

2.7 Glutathione reductase assay

Glutathione reductase (GR) was determined by measuring the reduction rate of oxidized glutathione (GSSG) with NADPH as enzyme substrate at 340 nm [12]. Activity of GR was defined as nmol of NADPH/min/106 of cells.

2.8 Glutathione-S-transferase assay

Glutathione-S-transferase (GST) was based on conjugation of –SH group of reduced glutathione with 1-chloro-2,4-dinitrobenzene (CDNB) [12]. Absorbance of the conjugate CDNB-glutathione was measured at 340 nm. Activity of GST was expressed as nmol of CDNB-glutathione conjugate/min/10^6 of cells.

2.9 Micronucleus test

CBMN was performed by the standard cytogenetic procedure with minor modifications regarding staining, as described previously [13]. Cells were stained with Giemsa (2%) in distilled water with three drops of NH_4OH for 9 min.

At least 1000 cells per each sample were analyzed. Monitored values included: frequency of micronuclei, micronucleus distribution and proliferation index. MN frequency was presented as a number of micronuclei per 1000 examined binuclear cells. Micronucleus distribution was acquired by scoring the binuclear cells containing one or more micronuclei. The proliferation index (PI), that represents a measure of the number of cell cycles that a cell population passes through, was calculated according to formula:

$$NDI = \frac{M1+2M2+3(M3+M4)}{N}$$

where M1-M4 represent the numbers of cells with 1–4 nuclei, respectively, and N is the total number of scored cells (IAEA, 2001).

The prepared material was observed and analyzed by light microscopy (Olympus BX51, Germany).

2.10 Statistical analysis

For comparison between the exposed and unexposed K562 cell line groups, ANOVA (single factor) and Student's *t* test (two-sample assuming equal variances) were used. For micronuclei and proliferation index, differences between untreated and treated groups were analyzed by Wilcoxon Matched Pairs Test and ANOVA, using STATISTICA Release 8. The statistical significance for all tests was set at $p < 0.05$.

3. Results

3.1 Effect of ELF-EMF on lipid peroxidation and activity of antioxidative enzymes in K562 cell line

The results related to the process of lipid peroxidation and activities of antioxidative enzymes are expressed as a percentage of the value obtained for the unexposed control K562 cell line groups.

As shown in Figure 1, ELF-EMF exposure did not induce significant increase in MDA level in any treated group. A small increase was noticed in the group exposed to 100 μT electromagnetic field for 24 h, however the increase was not statistically significant.

Figure 2 shows the main antioxidant and GSH dependent detoxifying enzymatic activities in ELF-EMF-treated K562 cells.

It is evident that extremely low-frequency exposure significantly increased activity of glutathione-S-transferase (Figure 2A).

Glutathione-reductase activities (Figure 2B) were also increased, except in the case of 40 μT electromagnetic field for 3 h where the activity of gluthation-reductase maintained a level similar to the unexposed control group.

A statistically significant increase in catalase (Figure 2C) activity was observed only in the experimental group exposed to 100 μT electromagnetic field for 24 h.

ELF-EMF treatment did not affect superoxide dismutase activity (Figure 2D), although a slight, but not statistically significant decrease in activity was observed in all exposed groups.

3.2 Effect of ELF-EMF on micronuclei on normal human peripheral blood lymphocytes

The MN frequency in both groups treated with 100 μT ELF-EMF was significantly lower compared to the untreated group (for 24 h treatment: p=0.24, and for 48 h treatment p=0.20; Wilcoxon test). The MN frequency in the group treated with 100 μT ELF-EMF for 24 h was slightly lower than the frequency in the group treated for 48 h, although without statistical significance (Figure 3).

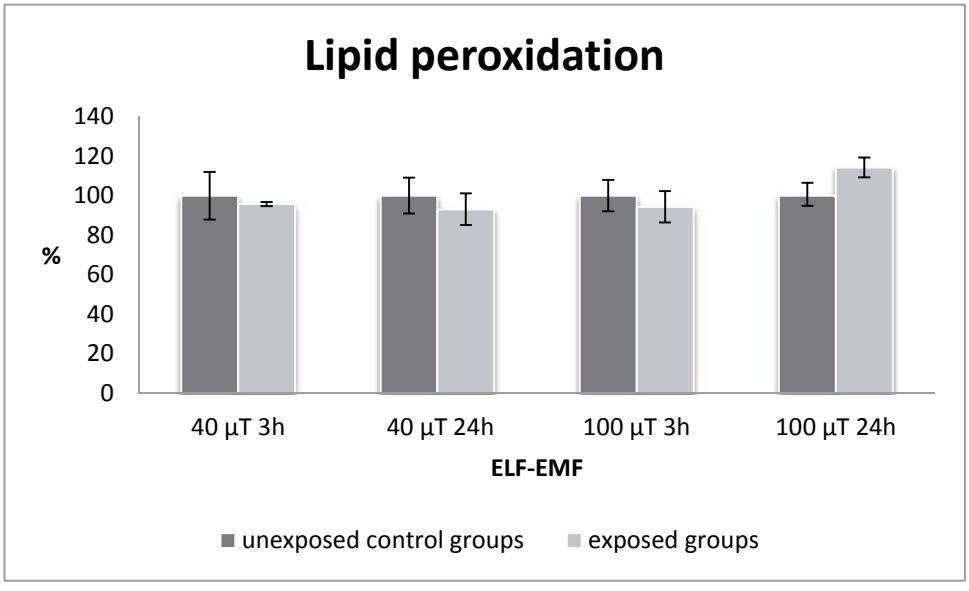

Figure 1. Effect of ELF-EMF on process of lipid peroxidation in K562 cell line. The results are expressed as a percentage related to the unexposed control cells (*$p < 0.05$ vs unexposed control cells).

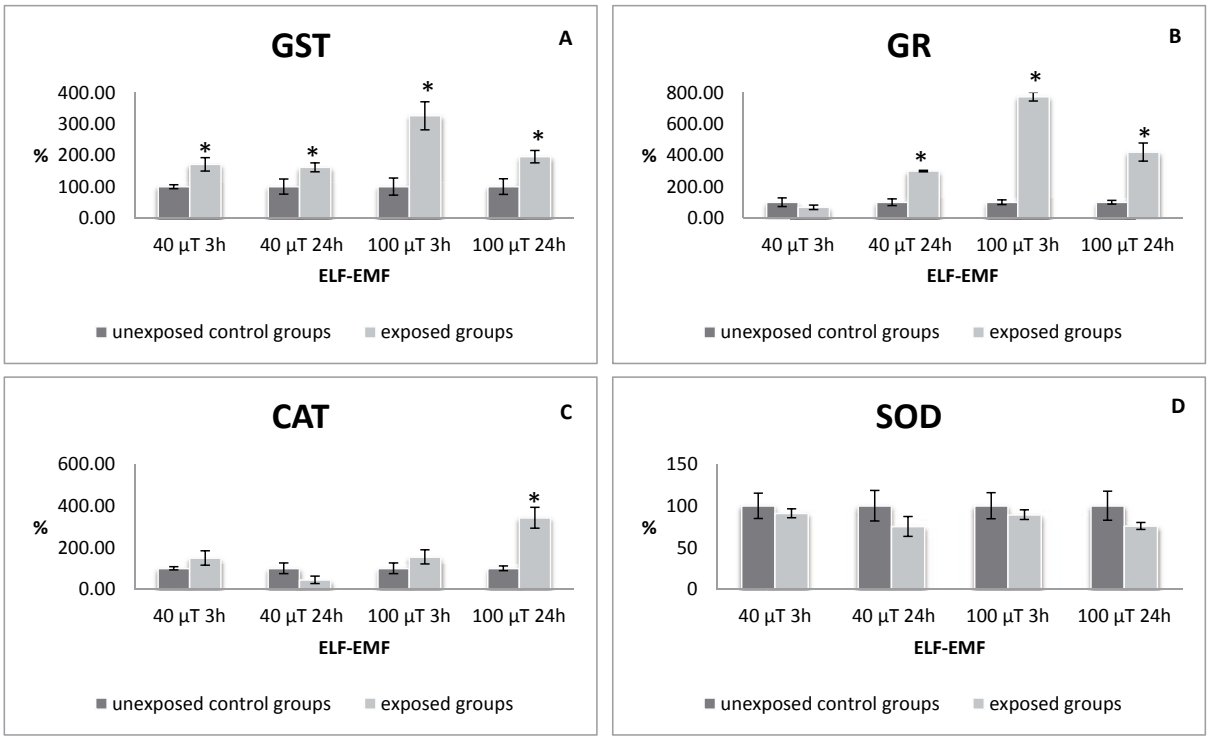

Figure 2. Effect of ELF-EMF on activity of antioxidative enzymes in K562 cell line. A–glutathione-S-transferase (GST); B-glutathione-reductase (GR); C–catalase (CAT) D -superoxide dismutase (SOD) The results are expressed as a percentage related to the unexposed control cells (*$p < 0.05$ vs unexposed control cells).

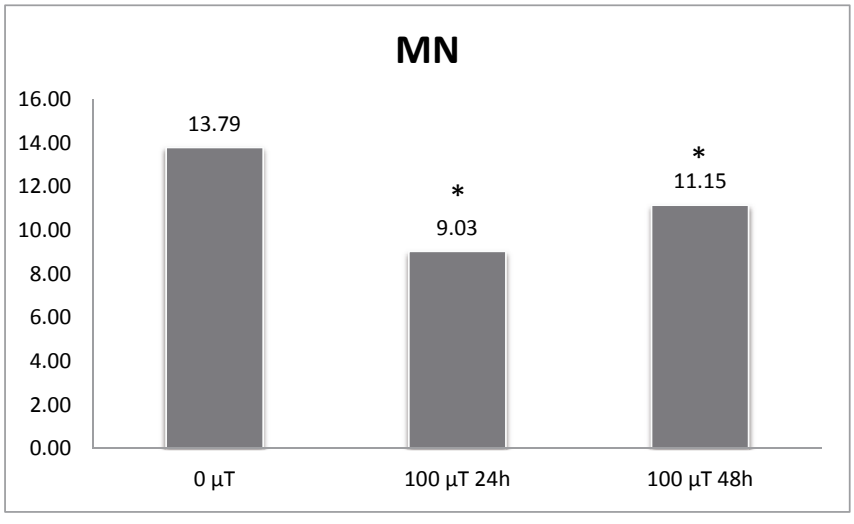

Figure 3. Effect of ELF-EMF on MN frequency in the untreated normal human lymphocytes and treated with 100 μT ELF-EMF for 24h and 48h. The values are presented as mean values; MN - the frequency of micronuclei.

3.3 Effect of ELF-EMF on proliferation normal human peripheral blood lymphocytes

The PI value in both groups treated with 100 µT ELF-EMF was significantly higher compared to the untreated group (for 24 h treatment: p=0.007, and for 48 h treatment p=0.028; Wilcoxon test). The PI value in the group treated with 100 µT ELF-EMF for 24 h was slightly higher than the value in the group treated for 48 h, although without statistical significance (Figure 4).

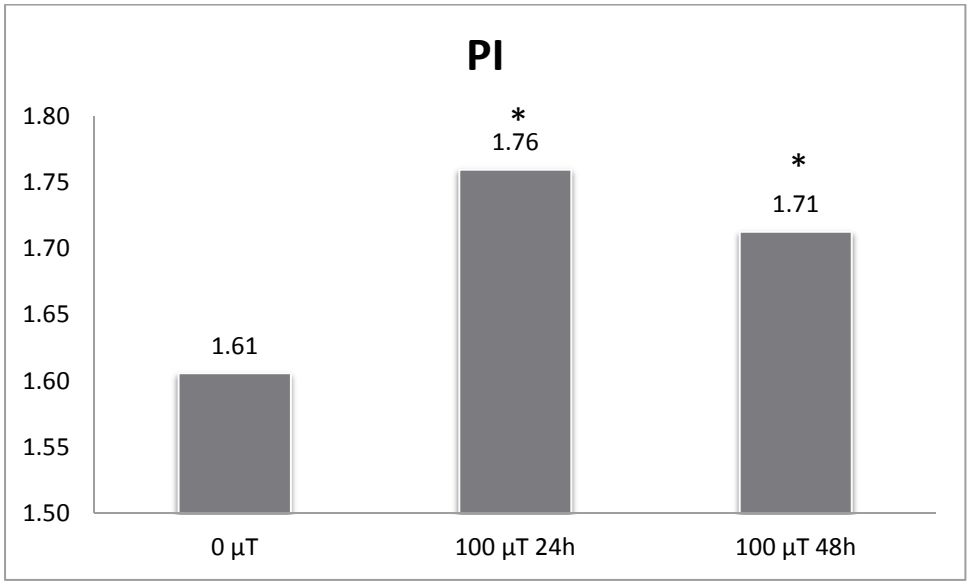

Figure 4. Effect of ELF-EMF on proliferation index in the untreated normal human lymphocytes and treated with 100 μT ELF-EMF for 24h and 48h. The values are presented as mean values; PI - proliferation index.

4. Discussion

One of the main features of cancer cells, when compared to the normal ones is a persistent pro-oxidative state that can lead to intrinsic oxidative stress. The enhanced ROS generation is described in several *in vitro* and *in vivo* cancer models, and strongly supports this condition. In fact, persistent oxidative stress in cancer cells induces a number of adaptive mechanisms, activating redox-sensitive transcription factors such as NF-kB and Nrf2, which causes an increase in the levels of certain antioxidant enzymes and GSH, anti-apoptotic growth factors such as BCL2 and MCL1, as well as inhibition of caspases which are important proapoptotic factors. Additionally, increased activity of thioredoxin reductase-1, translocates to the nucleus in response to oxidative stress, where it acts to maintain a reducing environment required for effective DNA binding of transcription factors and subsequent gene expression. All these processes allow cancer cells to live in conditions of increased oxidative stress and favor cancerogenesis [14].

Although a moderate increase of ROS may induce cell proliferation, excessive amounts of ROS can cause oxidative damage to lipids, proteins, and DNA, provoking oncogenic transformation, increased metabolic and mitochondrial activity, which results in apoptosis and cell death dysfunction. ROS participate simultaneously in two signaling pathways that have inverse functions in tumorigenesis, Ras-Raf-MEK1/2-ERK1/2 signaling and the p38 mitogen-activated protein kinases (MAPK) pathway. It is well known that Ras-Raf-MEK1/2-ERK1/2 signaling is related to oncogenesis, while the p38 MAPK pathway contributes to cancer suppression. Thus, ROS may not be an absolute carcinogenic factor or cancer suppressor [15].

Based on the results of the lipid peroxidation measurement in our experiment, we concluded that no significant increase in ROS levels is detected in K562 cells, at any time or magnetic flux density considered. Obtained results are in accordance with the results of Garip and Akan that ELF-EMF are not strong inducers of oxidative stress in tumor cells and act rather as a co-stressor, although additional studies are required [7].

Interestingly, ELF-EMF exposure increased the GST activity in all tested groups of K562 cell line. Elevated levels of GST are associated with increased resistance to apoptosis initiated by a variety of stimuli and have been reported in a large number of tumor types where they act as inhibitors of the p38 MAPK pathway. It is known that activation of MAPK signaling pathway paradoxically triggers a cell cycle arrest known as cellular senescence, contributing to tumor suppression [16]. Our results, elevated GST activities in all treated groups, indicate inhibition of the of MAPK signaling pathway, which could be one of the reasons why more apoptotic cells are missed after ELF-EMF exposure [17].

In all treated groups, except in the first one exposed to 40 µT EMF for 3 h, we detected an increased activity of GR, whose role is to maintain a high intracellular ratio of GSH/GSSG essential for the protection against oxidative stress. Additionally,

ELF-EMF exposure did not affect the activities of CAT or SOD either. Consequently, increase of lipid peroxidation was not detected. Increased GR activity could indicate the consequent more reduced cellular milieu which allows cell to scavenge efficiently the likely ELF-EMF mediated ROS over production, thus avoiding oxidative EMF-ELF induced cellular damage, as reviled by the absence of any significant effect on cell growth and viability [17]. Changes in the redox status were reported in neuroblastoma cells. These results suggest that a 50 Hz, 1 mT magnetic field modulates the redox status of the cells. Although no major oxidative damage was detected, positive modulation of antioxidant enzyme expression, as well as a significant increase in reduced glutathione level was observed, indicating a shift of cellular environment towards a more reduced state [5].

Simultaneous increase of CAT, GR and GST activity was detected only in the K562 cell line exposed to 100 μT EMF for 24 h. These results could be a consequence of the oxidative stress adaptation. In fact, at low concentrations of H_2O_2 and normal values of reduced glutathione (GSH), glutathione peroxidase has a key role in the detoxification of hydrogen peroxide, given that its Km value is less than Km values for CAT. Under conditions where H_2O_2 reaches high intracellular concentrations, catalase plays a key role in the elimination of H_2O_2. Our observations can be supported with the results of lipid peroxidation measurement; since slight increase could be noted in the group exposed to 100 μT electromagnetic field for 24 h. As a result of the elevated level of oxidative stress in this group increased number of apoptotic cell were seen [17]. Our assumption is that this degree of EMF-ELF exposure leads to the induction of ROS beyond protection level against apoptosis in cancer cells and exerts its cytotoxic effect. Namely, application of ELF-EMF once at the onset of hemin induction may have interfered with the differentiation process and led to a decrease in differentiation as a result of increased oxidative stress and consequent MAPK signal path activation, while daily ELF-EMF application overcame this effect by an increase in HSP levels. It is debated that the time course of application and the duration may be of a nonlinear character suggesting a "window" effect in duration of the field application [8].

Cancer cells are nearly always low in SOD activity [18]. In our experiments a small, but not statistically significant decrease of SOD activity was observed in all exposed groups. The explanation for this lack of effect could be explained by the fact that the method used was not sensitive enough to detect possible changes in the already low level of the enzyme activity.

The recent results of genotoxic potential of ELF-EMF on *in vitro* models are conflicting, and mechanism of interaction undefined. The former study suggests that due to low frequency, magnetic or electromagnetic fields can not transmit energy high enough to affect chemical bonds within DNA and thus directly damage DNA [19]. However, EMF could contribute to DNA damage through the action of free radical species [3,6]. Moreover, several hypotheses such as induction of electric currents, increased free-radical activity or acceleration of electron transfer in different enzymes and proteins have been proposed, but have not been proven yet [20]. It is important to mention that 100 μT magnetic flux density, used in our work, is amongst the higher flux densities used in laboratory studies aimed at elucidating the biological effects of ELF-EMF and recommended by the SCENIHR [2].

Our study showed that continuous 100 μT ELF-EMF during 24 h and 48 h treatment leads to a significant reduction in the MN frequency. The absence of genotoxic potential of ELF-EMF on a normal human lymphocytes is in agreement with the results of other authors on the same model [21]. In contrast, Focke *et al*. showed positive genotoxic effects of intermittent ELF-EMF on human fibroblast by Comet assay. At the same time the absence of oxidative DNA damage was noticed. It was presumed that these effects were caused by the magnetic rather than the electric field [22], as indicated by Brocklehurst *at al*., who emphasized perturbation of free-radical recombination by magnetic fields [23]. Ivancsits *et al*. showed that different results from studies on continuous and intermittent exposure may be due to a slow induction of DNA-repair processes when cells are exposed intermittently as evidenced by the appearance of single strand breaks (SSB) and double-strand DNA breaks (DSB) in the DNA of human fibroblasts [24]. But the question is whether SSB represented lesions were produced by EMF, or they were intermediates in the repair of other lesions such as oxidized bases [3]?

Anyway, recent study showed that, if induction of DNA-repair process occurs faster and more efficient during continuous exposure to ELF-EMF, as in our study, this phenomenon could possibly compensate even a stronger genotoxic attack. Intense and efficient DNA-repair occurs likely in the acute treatment what could possibly attributed to the reduced micronuclei incidence after 24 and 48 h treatment with ELF-EMF, as shown in our work, since a correlation between chronic exposure to ELF-EMF intensity 0.13-0.18 μT and 6 mutated genes for the DNA repair enzymes in children with acute leukemia is noticed [25]. Furthermore, reduction of the DNA damage in lymphocytes during 24 h treatment with ELF-EMF magnetic induction 100 μT is in accordance

with the increased values of antioxidative enzymes CAT, GR and GST during the same treatment.

Our study found that treatment with 100 μT ELF-EMF during 24 h and 48 h led to the increased cell proliferation. Literature data reveal various impacts of ELF-EMF on cell cycle. Some of them report stimulatory [26], some inhibitory effects [6,27] and some studies even showed the absence of effect on cell cycle distribution [22]. Lisi *et al*. considered that ELF-EMF exposure not only triggers a metabolic cellular response able to assure good survival to cells, but also has a differentiating potential, since they induce significant increase in the levels of the differentiation markers growth-associated protein-43 (GAP-43) and neuronspecific enolase (NSE) [28]. Crumpton *et al*. pointed out that the ELF-EMF could influenze cell proliferation and DNA damage through the action of free radicals in both normal and tumor cells [3]. In contrast, Focke *et al*. showed positive genotoxic effects but for the intermittent ELF-EMP on human fibroblast by Comet assay [22]. No less important is the fact that free radicals have pleiotropic effects which may vary from cytotoxic to mitogenic responses depending on the dose intensity, the duration of exposure, and the type of cell or tissue [29]. On the other hand, it has been demonstrated that low levels of reactive oxygen species trigger intracellular signals that involve the transcription of genes and lead to responses including proliferation [30]. Slightly elevated value of lipid peroxidation shown in our study in short-term treatment with 100 μT ELF-EMF could indicate a low level induced ROS and explain stimulating effect on the proliferation of cells.

Based on literature data and our results we can conclude that ELF-EMF is a weak stressor, which alone can or cannot generate physiological responses depending on the strength and time of EMF exposure. However, based on the study of Garip and Akan ELF-EMF as co-stressor can augment or dampen the effect of another weak stressor thus causing a significant outcome. Hence ELF-EMF may cause increased adverse effects on biological systems in more polluted areas acting as a co-stressor in oxidative stress production [7]. It is important to emphasize that the previous studies conducted on the same type of cells examined the impact of much stronger EMF (1-5 mT) which is up to 50 times higher than the field strength in our experiment. It should be noted that the biological effects and the strength and the time of exposure of magnetic fields and, based on a number of previously conducted studies, are not linearly dependent.

High intrinsic oxidative stress in cancer cell, often accompanied by increased antioxidant capacity, make these cells more sensitive than normal cells to an additional increase in ROS, either by exogenous agents that stimulate the production of ROS or by inhibition of antioxidant protection. Results of this study indicate that 50 Hz ELF-EMF of particular magnetic induction (40 μT and 100 μT) are week stressors which alone cannot generate enough ROS to induce process of lipid peroxidation in cancer cell line but strong enough to induce response of antioxidative defense system. In accordance to these results are the results about reduction of DNA damage and stimulative effect of ELF-EMF treatment on proliferation of normal human lymphocytes, obtained also in our study.

In conclusion, our experimental data indicate that ELF-EMF treatment increases the activity of antioxidative enzymes in tumor cells and decrease the DNA damage of normal human lymphocytes with stimulation of cell proliferation. We are aware that our results derive from a limited study, but obtained data suggest that cellular response to ELF-EMF might involve different strategies engaging antioxidant and probably DNA repairing enzymatic systems, that contributes to DNA damage in acute treatment, as shown in our study.

Acknowledgment

This work is supported by The Provincial Secretariat for Science and Technological Development of Vojvodina (grant number 114-451-2056/2011-01) and the Ministry of Education and Science, Republic of Serbia, project No 175056.

Authors declared no conflict of interest.

References

[1] Repacholi M.H., Greenebaum B. Interaction of static and extremely low frequency electric and magnetic fields with living systems: Health effects and research needs. Bioelectromagnetics, 1999, 20(3), 133-160

[2] Ahlbom A., Bridges J., de Seze R., Hillert L., Juutilainen J., Mattsson M.O., et al. Possible effects of electromagnetic fields (EMF) on human health - Opinion of the Scientific Committee on Emerging and Newly Identified

Health Risks (SCENIHR). Toxicology, 2008, 246(2-3), 248-250
[3] Crumpton M.J., Collins A.R. Are environmental electromagnetic fields genotoxic? DNA Repair (Amst), 2004, 3(10), 1385-1387
[4] Simko M. Induction of cell activation processes by low frequency electromagnetic fields. ScientificWorldJournal, 2004, 4 Suppl 2, 4-22
[5] Falone S., Grossi M.R., Cinque B., D'Angelo B., Tettamanti E., Cimini A., et al. Fifty hertz extremely low-frequency electromagnetic field causes changes in redox and differentiative status in neuroblastoma cells. Int J Biochem Cell Biol, 2007, 39(11), 2093-2106
[6] Wolf F.I., Torsello A., Tedesco B., Fasanella S., Boninsegna A., D'Ascenzo M., et al. 50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a redox mechanism. Biochim Biophys Acta, 2005, 1743(1-2), 120-129
[7] Garip A.I., Akan Z. Effect of ELF-EMF on number of apoptotic cells; correlation with reactive oxygen species and HSP. Acta Biol Hung, 2010, 61(2), 158-167
[8] Ayse I.G., Zafer A., Sule O., Isil I.T., Kalkan T. Differentiation of K562 cells under ELF-EMF applied at different time courses. Electromagn Biol Med, 2010, 29(3), 122-130
[9] Brocklehurst B., McLauchlan K.A. Free radical mechanism for the effects of environmental electromagnetic fields on biological systems. Int J Radiat Biol, 1996, 69(1), 3-24
[10] Vijayalaxmi, Obe G. Controversial cytogenetic observations in mammalian somatic cells exposed to extremely low frequency electromagnetic radiation: A review and future research recommendations. Bioelectromagnetics, 2005, 26(5), 412-430
[11] Srdjenovic B., Milic-Torres V., Grujic N., Stankov K., Djordjevic A., Vasovic V. Antioxidant properties of fullerenol C60(OH)24 in rat kidneys, testes, and lungs treated with doxorubicin. Toxicol Mech Methods, 2010, 20(6), 298-305
[12] Beutler E. Red cell metabolism: a manual of biochemical methods: Grune & Stratton; 1984.
[13] Mrdanovic J., Jungic S., Solajic S., Bogdanovic V., Jurisic V. Effects of orally administered antioxidants on micronuclei and sister chromatid exchange frequency in workers professionally exposed to antineoplastic agents. Food Chem Toxicol, 2012, 50(8), 2937-2944
[14] Trachootham D., Alexandre J., Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? Nat Rev Drug Discov, 2009, 8(7), 579-591
[15] Pan J.S., Hong M.Z., Ren J.L. Reactive oxygen species: A double-edged sword in oncogenesis. World Journal of Gastroenterology, 2009, 15(14), 1702-1707
[16] Townsend D.M., Tew K.D. The role of glutathione-S-transferase in anti-cancer drug resistance. Oncogene, 2003, 22(47), 7369-7375
[17] Savic M. Electromagnetic field intensity bias on functional ability of K526 cell line, Master's thesis. University of Novi Sad, Novi Sad, Serbia, 2010
[18] Oberley T.D., Oberley L.W. Antioxidant enzyme levels in cancer. Histol Histopathol, 1997, 12(2), 525-535
[19] McCann J., Dietrich F., Rafferty C. The genotoxic potential of electric and magnetic fields: an update. Mutat Res, 1998, 411(1), 45-86
[20] Winker R., Ivancsits S., Pilger A., Adlkofer F., Rudiger H.W. Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields. Mutat Res, 2005, 585(1-2), 43-49
[21] Scarfi M.R., Bersani F., Cossarizza A., Monti D., Zeni O., Lioi M.B., et al. 50 Hz AC sinusoidal electric fields do not exert genotoxic effects (micronucleus formation) in human lymphocytes. Radiat Res, 1993, 135(1), 64-68
[22] Focke F., Schuermann D., Kuster N., Schar P. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure. Mutat Res, 2010, 683(1-2), 74-83
[23] Brocklehurst B. Magnetic fields and radical reactions: recent developments and their role in nature. Chemical Society Reviews, 2002, 31(5), 301-311
[24] Ivancsits S., Diem E., Jahn O., Rüdiger H.W. Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields. Mechanisms of Ageing and Development, 2003, 124(7), 847-850
[25] Yang Y., Jin X.M., Yan C.H., Tian Y., Tang J.Y., Shen X.M. Case-only study of interactions between DNA repair genes (hMLH1, APEX1, MGMT, XRCC1 and XPD) and low-frequency electromagnetic fields in childhood acute leukemia. Leukemia & Lymphoma, 2008, 49(12), 2344-2350
[26] De Mattei M., Caruso A., Traina G.C., Pezzetti F., Baroni T., Sollazzo V. Correlation between pulsed electromagnetic fields exposure time and cell proliferation increase in human osteosarcoma cell lines and human normal osteoblast cells in vitro. Bioelectromagnetics, 1999, 20(3), 177-182

[27] Lange S., Richard D., Viergutz T., Kriehuber R., Weiss D.G., Simko M. Alterations in the cell cycle and in the protein level of cyclin D1, p21(CIP1), and p16(INK4a) after exposure to 50 Hz MF in human cells. Radiation and Environmental Biophysics, 2002, 41(2), 131-137

[28] Lisi A., Ledda M., Rosola E., Pozzi D., D'Emilia E., Giuliani L., et al. Extremely low frequency electromagnetic field exposure promotes differentiation of pituitary corticotrope-derived AtT20 D16V cells. Bioelectromagnetics, 2006, 27(8), 641-651

[29] Davies K.J. The broad spectrum of responses to oxidants in proliferating cells: a new paradigm for oxidative stress. IUBMB Life, 1999, 48(1), 41-47

[30] Dong-Yun S., Yu-Ru D., Shan-Lin L., Ya-Dong Z., Lian W. Redox stress regulates cell proliferation and apoptosis of human hepatoma through Akt protein phosphorylation. FEBS letters, 2003, 542(1), 60-64