Extremely low frequency magnetic field induces oxidative stress in mouse cerebellum

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Abstract. We have investigated whether extremely low frequency magnetic field (ELF-MF) induces lipid peroxidation and reactive oxygen species in mouse cerebellum. After exposure to 60 Hz ELF-MF at 2.3 mT intensity for 3 hours, there was a significant increase in malondialdehyde level and hydroxyl radical. ELF-MF significantly induced concomitant increase in superoxide dismutase without alteration in glutathione peroxidase activity. While glutathione contents were not altered, ascorbic acid levels were significantly decreased by ELF-MF exposure. These results indicate that ELF-MF may induce oxidative stress in mouse cerebellum. However, the mechanism remains further to be characterized.

Key words: Extremely low frequency magnetic field — Lipid peroxidation — Hydroxyl radical — Superoxide dismutase — Ascorbic acid

Abbreviations: ELF-MF, extremely low frequency magnetic field; GPx, glutathione peroxidase; GSH, glutathione; GSSG, oxidized disulphide; MDA, malondialdehyde; ROS, reactive oxygen species; SOD, superoxide dismutase.
coordination and certain types of learned motor behavior (Haines and Dietrichs 1989; Anderson and Steinmetz 1994). This region is one of a few sites in the CNS where the pattern of intrinsic connections is known in considerable detail. Oxidative stress in cerebellum may contribute to many kinds of diseases including cancer and neurodegenerative diseases (Chung et al. 2000; Salman et al. 2009).

Our working hypothesis is that ELF-MF can affect biological systems by induction of free radicals. It has been suggested that 50/60 Hz ELF-MF may prolong the lifetime of free radicals (Repacholi and Greenebaum 1999; Akdag et al. 2007) and increase their concentration in living cells (Jajte et al. 2002). The increased life span of free radical makes living system including brain susceptible to lipid peroxidation. It has been found that electric and magnetic fields can influence lipid peroxidation under certain experimental conditions (Harakawa et al. 2005), although reports on the subject are conflicting. To elucidate our working hypothesis, we investigate one of major lipid peroxidative markers, malondialdehyde (MDA), and antioxidant defense systems, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH) and ascorbic acid in mouse cerebellum following exposure to 60 Hz ELF-MF at 2.3 mT for 3 hours.

The experiments were performed on 20 male Balb/C mice. All mice were handled in accordance with National Institute of Health guidelines for the humane care of laboratory animals. Four to five week-old mice were maintained on a 12/12 h light/dark cycle with diet and water available ad libitum and were adapted for 2 weeks to these conditions before the experiment. Mice were assigned into two groups (sham group, n = 10; experimental group, n = 10). The experimental group mice were exposed to 2.3 mT, 60 Hz ELF-MF for 3 hours in the mouse cage. For the sham group, the similar experimental conditions (Harakawa et al. 2005), although reports on the subject are conflicting. To elucidate our working hypothesis, we investigate one of major lipid peroxidative markers, malondialdehyde (MDA), and antioxidant defense systems, such as superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione (GSH) and ascorbic acid in mouse cerebellum following exposure to 60 Hz ELF-MF at 2.3 mT for 3 hours.

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The SOD enzyme activity was measured according to the method described by Bellissimo et al. (2001) with slight modification. Cerebellum was homogenized in PBS 0.05 M (pH 7.4) in buffer solution (1 mg/5 μl). Each homogenate was centrifuged at 10,000 × g (4°C) for 20 min, and the supernatant was carefully separated. After homogenization, 20 μg of cerebellum was mixed with 0.5 ml of a solution composed by 0.05 mol/L phosphate buffer (PBS), pH 7.0 containing hydroxylamine (0.2 mmol/l) and hypoxanthine (0.2 mmol/l). The reaction was started by addition of 1 ml of a solution, composed by 1.25 mM/mL xanthine oxidase, 10⁻⁴ M EDTA dissolved in 20.8 mmol/l potassium buffer, pH 7.0 and Na₂B₄O₇ (15 mmol/l). This mixture was incubated for 20 min at 37°C without shaking. Finally, 1.0 ml of the N-1-naphthylethlenediamine (5 μg/ml) and sulfanilic acid (300 μg/ml) diluted in 25 % acetic acid was added. The final mixture was allowed to stand for 20 min at room temperature and the optical absorption was measured at 550 nm. A unit of enzyme activity (U) is defined as the quantity of protein responsible for the inhibition of 50% of this reaction and the activity was expressed as U/mg of protein.

The GPx activity was measured according to the method described by Bellissimo et al. (2001) with slight modification. Cerebellum was homogenized in PBS 0.05 M (pH 7.4) in buffer solution (1 mg/5 μl). After homogenization, 30 μg of protein was added to 500 μl of PBS pH 7.0 containing 10⁻³ mol/l reduced glutathione, 2 units of yeast glutathione reductase and 2 × 10⁻⁴ NADPH. After 20 min at 37°C, the reaction was initiated by the addition of t-butyl-GPx to a final concentration of 10⁻³ mol/l, under constant agitation. The oxidation of NADPH was calculated using extinction coefficient for NADPH of 6.22 × 10³ at 340 nm and the reaction was made for 5 min. One enzyme unit was defined as 1 μmol/l NADPH/mU per mg of protein.
Induction of oxidative stress by magnetic field

Glutathione level immediately was measured from dissected tissues using a minor modification of the method described by Reed et al. (1980). Briefly, each sample of the acidified supernatant was added to the internal standard (1 mmol/l cysteic acid) in 0.88 mol/l iodoacetic acid. Excess sodium hydrogen carbonate was added to the reaction to precipitate sodium perchlorate. Subsequently, 0.5 ml of an alcoholic solution of 1.5% (v/v) 2,4-dinitrofluorobenzene was added to each sample, and the samples were incubated for 4 h. Diethyl ether (1.0 ml) was added, and the samples were shaken and centrifuged (20 min, 2000 × g, room temperature). The residual aqueous phase containing derived glutathione was separated and analyzed by HPLC. The separation of 5-carboxymethyl glutathione was carried out at room temperature with a flow rate of 1.2 ml/min. Chromatographic separation of derivatives was performed by injecting samples (10 μl) of the aqueous phase onto a Spherisorb S-5 amino ODS column. Glutathione was subsequently detected using a UV detector at 365 nm. Glutathione were quantified in relation to the internal standard (cysteic acid).

Ascorbic acid concentration (mg/dl) was measured by the photometric method. Ascorbic acid in sample was oxidized by Cu$^{2+}$ to form dehydroascorbic acid, which reacts with acidic 2,4-dinitrophenylhydrazone to form a red bis-hydrazone, which was measured at 520 nm. Two hundred μl of freshly prepared metaphosphoric acid were added to sample 1 ml and concentrated for 10 min at 2500 rpm. Two hundred microliters of dinitrophenylhydrazine-thiourea-copper sulfate (DTCS) reagent was added to 300 μl of the clear supernatant, standard and blank. The tubes were incubated in a water bath at 37°C for 3 h. After cooling 2 ml of cold sulfuric acid (12 mol/l) was added. The absorbance at 520 nm was determined against the blank.

All data were expressed as mean ± SEM. Statistical analysis was performed using unpaired t-test. Differences were considered significant when $p < 0.05$.

As a marker for oxidative stress, we assayed MDA for lipid peroxidation and hydroxyl radical for production of reactive oxygen species (ROS) (Fig. 1). MDA level was significantly increased ($p < 0.05$) in the cerebellum of mouse exposed to 60 Hz ELF-MF for more than 3 h compared to sham group when mice were exposed to ELF-MF (2.3 mT) for different times (1–5 h) (Fig. 1A). Therefore, we fixed the exposure period at 3 h in this study. Level of hydroxyl radical was assayed by quantifying 2,3-DHBA and 2,5-DHBA (Fig. 1B).

**Figure 1.** Effect of ELF-MF on production of MDA and hydroxyl radical in mouse cerebellum. **A.** Mice were exposed to 60 Hz ELF-MF (2.3 mT) for different times (1 h–5 h). MDA production was significantly increased in mouse cerebellum exposed to ELF-MF for 3 h compared to sham group. **B.** The production of hydroxyl radical was measured by assaying 2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-dihydroxybenzoic acid (2,5-DHBA), using salicylic acid conjugation methods. After ELF-MF exposure, 2,5-DHBA level was significantly increased. Each column indicated the mean ± SEM in seven to eight mice. * indicates values which are significant from control group at $p < 0.05$. ELF-MF, extremely low frequency magnetic field.
using HPLC-ECD system after ELF-MF exposure. The exposure significantly raised the level of 2,5-DHBA \((p < 0.05)\) but not 2,3-DHBA.

We also measured major enzymatic or non-enzymatic antioxidants in mouse cerebellum, including SOD, GPx, GSH and ascorbic acid. SOD and GPx activities were assayed to evaluate the effect of ELF-MF exposure on enzymatic defense systems against oxidative stress (Fig. 2). SOD activity was significantly increased in mouse cerebellum \((p < 0.05)\), while there was no significant alteration in GPx activity (Fig. 2A). The alteration of GSH and ascorbic acid level after ELF-MF exposure was observed as non-enzymatic defense components. GSH contents were not significantly different from sham controls, but ascorbic acid levels were significantly decreased by exposure to 60 Hz ELF-MF for 3 h \((p < 0.05, \text{Fig. 2B})\).

Exposure to ELF-MF comes from many sources, like high voltage transmission lines and electric appliances such as TV monitors, radios, hair dryers and electric blankets, because sources with high voltage and strong currents produce strong electromagnetic fields. The strength of ELF-MF weakens with increasing distance from the source. In a survey of ELF-MF around almost different ELF-MF household appliances, weak levels from 0.03 to 2.9 μT were measured in a typical user distance (Delpizzo 1990). However, the ELF-MF generating from transmission lines, transportation and industrial work-place was strong up to 0.1–50 mT (Stern 1987). The strength of ELF-MF we exposed to animals in this study was comparable to the occupational exposure predominant from working near industrial equipment using high currents.

This study clearly demonstrated that the levels of hydroxyl radical and MDA were significantly increased in mouse cerebellum following exposure to 60 Hz ELF-MF at 2.3 mT for 3 hours. As enzymatic defense systems against oxidative stress, SOD activity was significantly increased in mouse cerebellum, while there was no significant alteration in GPx activity. Among non-enzymatic antioxidants, GSH contents were not significantly changed, but ascorbic acid levels were significantly decreased in ELF-MF-exposed mice.

Our biochemical data of MDA and hydroxyl radical measurement were consistent with our previous chemilu-

![Figure 2](image-url)

**Figure 2.** Effects of ELF-MF on enzymatic (A) and non-enzymatic (B) antioxidant activities in mouse cerebellum. The exposure to ELF-MF significantly increased SOD activity in mouse cerebellum while it failed to change in GPx activity. There was a significant decrease in the level of ascorbic acid in cerebellum after ELF-MF while the level of glutathione was not altered. Each column indicates the mean ± SEM of in seven to eight mice. * indicates values which are significant from control group at \(p < 0.05\). ELF-MF, extremely low frequency magnetic field; SOD, superoxide dismutase; GPx, glutathione peroxidase.
minescence data (Lee et al. 2004). After exposure to ELF-MF, MDA levels were increased in guinea pig liver tissue (Canseven et al. 2008) and the lipid peroxidative process and H₂O₂ concentration were slightly increased in heart tissue (Goraca et al. 2010). Especially, an increase in MDA level was found in rat brains exposed to long-term ELF-MF (Akdag et al. 2010). In addition, ELF-MF-enhanced cell proliferation was suppressed by radical scavengers (Katsir and Parola 1998), indicating that ELF-MF is involved with free radical reaction in organism. MF exposure indeed induced DNA degradation and potentiated the activity of oxidant radicals, which could be diminished by the presence of an antioxidant (Li and Chow 2001). Combined with these previous findings, our observations have suggested that exposure to ELF-MF are among the effective conditions on the formation of free radicals and lipid peroxidation in mouse cerebellum.

Cells have developed an enzymatic antioxidant pathway against ROS which are generated during oxidative metabolism: firstly, the dismutation of superoxide anion (O₂⁻) to hydrogen peroxide (H₂O₂) catalyzed by SOD; and secondly, the conversion of H₂O₂ to H₂O by GPx or catalase (de Haan et al. 1995; Vazquez-Medina et al. 2006). Any increase in SOD catalytic activity produces an excess of H₂O₂ that must be efficiently neutralized by GPx. The activity of first and second step antioxidant enzymes must, therefore, be balanced to prevent oxidative damage in cells, which may contribute to various pathological processes (Sun and Chen 1998). Disequilibrium between SOD and GPx activity ratio could represent a marker of oxidative stress in cells (Mehta and Flora 2001). Therefore, an increased SOD activity in mouse cerebellum might represent an adaptive response to a higher superoxide ions production following exposure to 60 Hz MF.

Considering the roles of GPx as an antioxidant enzyme in oxidative stress, our results lead us to the hypothesis that where ELF-MF may act on the nonenzymatic defense system such as GSH. Although relatively resistant to 'spontaneous oxidation', GSH reacts rapidly and nonenzymatically with hydroxyl radical, and with dinitrogen trioxide (N₂O₃) and peroxynitrite (Kurozumi et al. 2005). In addition to its action as a chemical antioxidant, GSH also acts in the enzymatic first line antioxidant defense as a co-factor in GPx-mediated reduction of peroxides, also resulting in formation of the oxidized disulphide, GSGG. No significant alteration of GSH by ELF-MF exposure has indicated that the chemical reaction requiring the electron transfer between ROS and GSH or its derivatives may not be influenced by ELF-MF stimuli.

Among non-enzymatic antioxidants, ascorbic acid levels were significantly decreased in ELF-MF-exposed group in this study. Recent studies also revealed that ascorbic acid acts against oxidative stress in the CNS (Rodriguez-Martinez et al. 2004; Zaidi and Banu 2004). Ascorbic acid served as an effective antioxidant against restraint stress induced-pro-oxidant status and increased the anti-oxidant enzyme activity in rat brain (Zaidi and Banu 2004). It also decreased 3-propionic acid-induced oxidative stress in rat brain (Rodriguez-Martinez et al. 2004). After exposure to ELF-MF, the levels of lipid peroxide in rats treated with ascorbic acid were lower than those in the sham group (Harakawa et al. 2005). The present findings indicated that decreased level of ascorbic acid might be involved in ELF-MF-induced lipid peroxidation.

This study has demonstrated that ELF-MF may be a stimulus to be involved in free radical generation. The findings have also revealed that lipid peroxidation and antioxidant defense system such as SOD and ascorbic acid in the cerebellum was modulated by ELF-MF exposure. This is a meaningful observation for elucidation of molecular mechanism behind the process of ELF-MF in connection with oxidative stress. Further studies are needed to elucidate how ELF-MF can affect biological system in connection with oxidative stress.

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References


Canseven A. G., Coskun S., Seyhan N. (2008): Effects of various extremely low frequency magnetic fields on the free radical processes, natural antioxidant system, and respiratory burst
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http://dx.doi.org/10.1016/j.pediatrneurol.2008.11.020
http://dx.doi.org/10.1111/j.1471-4159.2007.05174.x

http://dx.doi.org/10.1016/S1567-5394(01)00152-9
http://dx.doi.org/10.1007/BF02255928


http://dx.doi.org/10.1289/ehp.8776221

http://dx.doi.org/10.1016/S1567-5394(01)00152-9
http://dx.doi.org/10.1007/BF02255928


http://dx.doi.org/10.1289/ehp.8776221

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