Effects of extremely low frequency magnetic field on the antioxidant defense system in mouse brain: a chemiluminescence study

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Abstract

Among the putative mechanisms, by which extremely low frequency (ELF) magnetic field (MF) may affect biological systems is that of increasing free radical life span in organisms. To test this hypothesis, we investigated whether ELF (60 Hz) MF can modulate antioxidant system in mouse brain by detecting chemiluminescence and measuring superoxide dismutase (SOD) activity in homogenates of the organ. Compared to sham exposed control group, lucigenin-initiated chemiluminescence in exposed group was not significantly increased. However, lucigenin-amplified t-butyl hydroperoxide (TBHP)-initiated brain homogenates chemiluminescence, was significantly increased in mouse exposed to 60 Hz, MF, 12 G for 3 h compared to sham exposed group. We also measured SOD activity, that plays a critical role of the antioxidant defensive system in brain. In the group exposed to 60 Hz, MF, 12 G for 3 h, brain SOD activity was significantly increased. These results suggest that 60 Hz, MF could deteriorate antioxidant defensive system by reactive oxygen species (ROS), other than superoxide radicals. Further studies are needed to identify the kind of ROS generated by the exposure to 60 Hz, MF and elucidate how MF can affect biological system in connection with oxidative stress.

Keywords: Extremely low frequency magnetic field; Chemiluminescence; t-Butyl hydroperoxide; Lucigenin; Superoxide dismutase; Reactive oxygen species

1. Introduction

In modern society, humans are commonly exposed to magnetic field (MF) including extremely low frequency magnetic field (ELF-MF), which is generally produced by power lines and many kinds of electric appliances. Not only experimental but also epidemiological data suggest, that there is an association between ELF-MF exposure and the increased incidence of certain types of tumor, particularly leukemia and brain cancer [1–3]. Those epidemiological data could be elucidated by the radical pair mechanism. The radical pair mechanism was suggested by Brocklehurst et al. [4] as a possible route, whereby a magnetic field of environmental strength might affect a biological system.

One of the mostly discussed contemporary problems is if ELF-MF can affect biological systems [5–7]. Increasing numbers of papers concerning this topic have been published. Lai and Singh [8] reported, that acute exposure to a 60 Hz, MF caused DNA strand breaks in the animal brain cells. However, there was contrary publication that neither ELF nor static electric or magnetic fields have a clearly demonstrated potential to cause genotoxic effects [9].

Lalo et al. [10] and Kabuto et al. [11] suggested that steady MF could accelerate lipid peroxidation. It was also reported that 60 Hz, MF increased the phorbol...
12-myristate 13-acetate (PMA) induced oxidative burst in neutrophils [12]. Fiorani et al. [13] reported that MF (50 Hz, 0.5 mT) increased the damage in an oxidative stressed rabbit erythrocyte system. These reports suggest that MF affect biological systems by prolonging the life of free radicals in the systems. Excess oxygen free radicals induce lipid peroxidation, especially in brain, which is very vulnerable to free radical insults because it contains high concentrations of easily peroxidizable fatty acid. Thus, the living organism has defensive systems against free radicals, such as the production of antioxidant enzymes. Among these antioxidants, superoxide dismutase (SOD) is critically important in brain. Recently the role of SOD to external stress has received much attention, especially in connection with ROS induced cellular damages [14].

Chemiluminescence (CL) is very useful to characterize oxygen free radicals-induced lipid peroxidation, since it has high sensitivity and specificity and detects unstable oxygen species [15]. In this study, we investigated whether 60 Hz, MF induces oxidative stress in mouse brain using pro-oxidant, tertiary butyl hydroperoxide (TBHP) and chemiluminescence enhancer, lucigenin.

2. Materials and methods

2.1. Animals

All mice were handled in accordance with National Institute of Health guidelines for the humane care of laboratory animals. Balb/c mouse (Seoul National University Laboratory Animal Center, Seoul, Korea) 4–5 weeks of age 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.

2.2. Magnetic field exposure system

The 60 Hz, MF was produced by 1 m² Helmholtz coil with winding embedded in an open wooden rectangular frame. Each coil was wound 100 turns with 1 mm-copper wire and was connected in a series connection to 220 V AC power supply via variable transformer. Each winding was split allowing the current to flow in the same sense through each half of the winding (field aiding). These coils produced a 60 Hz magnetic field set to 12 G r.m.s. amplitude (Lake Shore Model 410) in the horizontal direction at the center of the system. The mouse cage was made by non-magnetic material and was separated with coils in order to avoid any vibration or heating. Sham exposed mice were placed in the same location with the coils turned off and were exposed to the local ambient geomagnetic field ($B_H = 0.18$, $B_V = 0.22$, $B_T = 0.23$ and $B_{AC} < 0.02$ G).

2.3. Photon detection system

The CL was measured in a scaled dark box of the Chemiluminescence Analyzing System (Fig. 1). This system contains a photomultiplier tube (CPM photon counting module Type MP 962, Perkin–Elmer), Universal Counter (Hewlett-Packard 53131A, 225 Hz), 64 bit IBM personal computer. In order to inject TBHP or lucigenin into sample chamber without influence of external light, injection syringe is designed for being capable of manipulation outside the dark box. The dark box is covered with darkroom stuff in order to avoid the influence of external light.

2.4. Measurements of chemiluminescence

Seven different mice were sacrificed by decapitation after 3 h exposure to 60 Hz, MF, 12 G and sham exposure, respectively. Brains were rapidly removed on ice. Whole brains were homogenized in 5 volumes (w/v) with 0.05 M phosphate buffer solution (PBS, pH 7.4). We took 0.7 ml of the brain homogenates and laid the sample in absolute dark chamber for 10 min. Then we counted photon emission from the sample every 0.1 s interval at 37 °C under normal atmospheric condition [16]. At the 180 s point, we injected 1 ml lucigenin (0.05 mM) to the sample. At the 360 s point, we injected 1 ml of 75 mM TBHP (Sigma Co., USA). Chemiluminescence from brain homogenates was continuously measured for total 720 s. Integrating the area under the curve and subtracting dark counts from it, we calculated the total amount of CL. The assay was performed in duplicate for each sample and was expressed as CL counts per s.

2.5. Measurements of SOD enzyme activity

For measurement of SOD enzyme activity [17], seven different mice were also sacrificed by decapitation in sham exposure group and test group, respectively. Brain

![Fig. 1. The chemiluminescence analyzing system. PMT is abbreviation of photomultiplier tube.](image)
homogenates were centrifuged at 10,000 g (4 °C) for 20 min and the supernatant was carefully separated. After homogenization, 10 μg of whole brain protein was mixed with 0.5 ml of a solution composed by 0.05 M PBS, pH 7.0 containing hydroxylamine (0.2 mM) and hypoxanthine (0.2 mM). The reaction was started by addition of 1 ml of a solution, composed by 1.25 mU/ml xanthine oxidase, 10⁻⁴ M EDTA dissolved in 20.8 mM potassium buffer, pH 7.0 and Na₂B₄O₇ (15.6 mM). This mixture was incubated for 30 min at 37 °C without shaking. Finally, 1.0 ml of the N-1-naphthylethylenediamine (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.

2.6. Statistical analysis

Analysis of variance was used to establish the statistical significance of differences among groups. If significant differences among groups were obtained using the analysis of variance, Duncan’s multiple range test was used to differentiate differences between groups. Differences were considered significant if $p < 0.05$.

3. Results

Fig. 2 shows one case of the measurements of the photon counts from the homogenated mouse brain sample. Before the 0 s the average dark counts without the sample is recorded for reference purposes.

\[ N_{\text{dark}} = 9.1 \pm 0.51 \text{ (counts per second)}, \]

where ± 0.51 is the standard deviation.

In the first 180 s (0–180 s) the average photon counts from homogenated mouse brain alone is recorded in time and we compared the control with the test group (with MF):

\[ N_{\text{control}}(\text{brain}) = 11.3 \pm 1.1 \text{ (counts per second)}, \]
\[ N_{\text{test}}(\text{brain}) = 11.3 \pm 0.82 \text{ (counts per second)}, \]

where the standard deviations are calculated over the seven independent experiments.

At the 180 s the lucigenin (LC) is injected to the brain sample and the photon counts increase as:

\[ N_{\text{control}}(\text{LC}) = 15.3 \pm 1.0 \text{ (counts per second)}, \]
\[ N_{\text{test}}(\text{LC}) = 16.3 \pm 0.9 \text{ (counts per second)}. \]

At the 360 s the TBHP is injected to the lucigenin added mouse brain sample, and chemiluminescence increases by a large amount:

\[ N_{\text{control}}(\text{LC + TBHP}) = 18.4 \pm 1.0 \text{ (counts per second)}, \]
\[ N_{\text{test}}(\text{LC + TBHP}) = 22.2 \pm 2.4 \text{ (counts per second)}. \]

The test group emits more photons by about 20% than the control group.

Fig. 3 shows the results of the lucigenin-induced chemiluminescence counts (Photon counts difference between signal and dark during 180 s). The measured counts in the sham exposed control group is shown by (–)-mark and those of the test group with 60 Hz, MF is shown by (*)-mark line.

![Fig. 2. An example of photon counts from the homogenated mouse brain. The (+)-mark line (before 0 s) shows dark counts without sample. During the first 180 s (0–180 s) the sample is only the mouse brain homogenate. At 180 s, lucigenin is injected and TBHP is injected at 360 s. The photon counts in the sham exposed control group is shown by (·)-mark and those of the test group with 60 Hz, MF is shown by (*)-mark line.](image-url)
average total counts for the sham control group and the 60 Hz, MF exposed group are, respectively,

\[ N_{\text{control}}(\text{LC}) = 6.2 \pm 1.0 \text{ (counts per second)}, \]
\[ N_{\text{test}}(\text{LC}) = 7.2 \pm 0.9 \text{ (counts per second)}, \]

and there is no significant difference between them.

Fig. 4 shows one of our main results. The lucigenin amplified TBHP-initiated chemiluminescence counts show significant differences between the two groups: The test group exposed to 60 Hz, MF for 3 h emits significantly more photon than the sham exposed control group.

\[ N_{\text{control}}(\text{LC} + \text{TBHP}) = 9.3 \pm 1.8 \text{ (counts per second)}, \]
\[ N_{\text{test}}(\text{LC} + \text{TBHP}) = 13.1 \pm 2.4 \text{ (counts per second)}. \]

There is significant difference between them \( (p < 0.01) \).

Fig. 5 shows that the SOD activity is significantly increased by the magnetic fields exposure \( (p < 0.05) \). The test group is exposed to the 12 G, 60 Hz, MF for 3 h, and the control group is sham-exposed.

4. Discussion

It is found, that there are no significant differences between the chemiluminescence of the 60 Hz, MF exposed mouse brain homogenates and the sham exposed ones before and after the lucigenin is injected at the 180 s, even though there are large increase in the photon counts after the lucigenin is injected. Lucigenin-initiated chemiluminescence is indeed an effective monitor of superoxide radical generation [18] as lucigenin is a standard chemiluminescence probe, which produces low levels of light \((450-500 \text{ nm})\) on reaction with intracellular oxygen free radicals, being particularly sensitive to superoxide radicals. In this experiment, we did not find any significant change of lucigenin-initiated CL following exposure to 60 Hz, MF. This implies that the quantities of superoxide radicals are not significantly increased for 3 h exposure to 60 Hz magnetic fields compared with the sham exposed control group. However, in the case of lucigenin-amplified TBHP-initiated chemiluminescence, there is a significant increases in the photon counts of the 60 Hz, MF-exposed groups compared with the sham exposed control group. TBHP-initiated CL is an effective on-line indicator of lipid peroxide generation [19,20] and has been used to detect endogenous antioxidant activity in liver and heart [21,22]. Thus it is inferable that 60 Hz, MF may deteriorate endogenous antioxidant defensive system.

In endogenous antioxidant system, SOD is widely distributed and plays a critical role within mammalian organism. SOD has a pivotal role especially in protecting brain against the damaging effects from superoxide radicals [23]. It is considered that SOD at least transiently increases in response to oxidative stress in biological systems as a defense. In our experiment, SOD activity level in mouse brain is significantly increased following exposure to 60 Hz, 12 G, MF for 3 h. Increase in SOD activity may represent a compensatory reaction against oxidative stress and we consider that highly activated SOD accounts for insignificant change of lucigenin-initiated CL, which mainly indicates superoxide radicals generation. Thus we infer that 60 Hz,
MF-induced deteriorating effect on antioxidant defensive system is contributed by other reactive oxygen species, rather than superoxide radicals. This is a meaningful observation for elucidation of molecular mechanism behind the process of 60 Hz, MF in connection with oxidative stress. However, the activity measurements of other antioxidant enzymes such as peroxidase, catalase, glutathione reductase are needed to elucidate the precise mechanism [24].

Our observation that 60 Hz, MF exposure augments the TBHP-initiated chemiluminescence is consistent with the results of biochemical data of MDA measurement data which also indicates that 60 Hz, MF exposure induces lipid peroxidative stress [25]. In addition, it was reported that ELF MF enhanced cell proliferation was suppressed by radical scavengers [26]. This report implies ELF MF is involved with free radical reaction in organism. Recently Li et al. [27], reported that in vivo system MF exposure indeed induced DNA degradation and this deleterious effect could be diminished by the presence of an antioxidant. In vitro test, they also showed that MF could potentiate the activity of oxidant radicals. These findings are in accordance with our observation of 60 Hz, MF exposure augments the TBHP-initiated chemiluminescence with concomitant increase of SOD activity in mouse brain.

In conclusion, these results suggest that 60 Hz, 12 G MF could deteriorate antioxidant defensive system by other reactive oxygen species other than superoxide radicals. Further studies are needed to investigate the kinds of ROS production following exposure to 60 Hz, MF to elucidate how MF can affect biological system in connection with oxidative stress.

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References


