Evaluation of Biological Effects of Magnetic Fields  
-from static to intermediate frequency-

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Abstract  
Mutagenic potential of various magnetic fields were investigated. Slight mutagenic and co-mutagenic potential were observed in strong static magnetic field above 2T while lack of effect found in other exposure conditions. This finding was slight effective on anti-tumor treatment in L1210 bearing BDF1 mice as a possible application in medicine.

Keywords : magnetic fields, biological effects, mutagenicity, medical application

1. Introduction  
Progress of technology is capable to use electric, magnetic and electromagnetic field (EMF) in various purposes. For example, static magnetic field (SMF) are used for levitation and propulsion of MagLev train car, SMF and intermediate frequency (IF) magnetic fields are used for magnetic resonance imaging(MRI), several tens kHz intermediate frequency (IF) magnetic fields are used for induction heating of cooking pan, etc. Also, many electrical devices generate various magnetic fields in results of using electric current as their power source, such as high-power inverter for electric train. These technologies bring us large benefits, however it is also necessary to evaluate its health risk to avoid confusion in society because of lack of possible health effect of EMF. Recently, WHO published two environmental health criteria monographs for static fields (No. 232, in 2006) [1] and time-varying (below 100 kHz) extremely low frequency fields (No. 238, in 2007) [2]. In these monograph, lack of basic knowledge was still pointed out. On the other hand, if biological effects of MFs exist, study of application in medicine should be promoted.

2. Materials and Methods

2.1 Magnetic field exposure  
Superconducting magnet was used to expose up to 5 T (tesla) static magnetic field [3]. Helmholtz’s coil was used to expose 50 Hz, up to 40 mT magnetic field [4]. These exposure apparatuses were located in constant temperature room and maintain exposure space at 24, 30 or 37 +/- 1 °C, respectively. Meritt coil was used to expose 21 kHz, up to 3.9 mT magnetic field [5]. Temperature, humidity and concentration of CO₂ was controlled using an incubator that made by resin that was located inside of the coil at 37 +/- 1 °C.

2.2 Mutation assays  
For Ames test, Salmonella typhimurium TA98 and TA100 were used. Cultured bacterial cells were poured on minimal glucose agar plates with a trace of histidine and biotin. These test plates divided into two groups and one group was incubated under magnetic field while the other group was incubated without magnetic field as control. After 48 hours, revertant colonies were scored.

For yeast mutation assay, 0.1 ml of cell suspension was mixed with molten soft agar (0.6 % Bacto-agar, 0.5 % NaCl) and poured on to low lysine synthetic complete plate for detecting point mutation frequency on lys 1-1. 0.1 ml of 1/100 diluted cell suspension was mixed with molten soft agar and poured on to low arginine synthetic complete plate for detecting gene conversion frequency on ARG4 allele (between arg 4-4 and arg 4-17).
For mouse lymphoma assay (MLA), L5178Y tk+/− 3.7.2C cell was used. Cells were inoculated in a T-25 flask filled with 5 ml of RPMI1640 medium with 10% horse serum (2.5x10^5 cells/ml) and were exposed to magnetic field for 48 hr in 5% CO₂ at 37°C with single dilution after 24 hr. Unexposed control cells were incubated in a conventional incubator. After exposure period, plate efficiency and frequency of 5-trifluoro thymidine (5-TFT) resistant cells as tk−/− mutant was determined.

For in vivo micronucleus test, BALB/c mice were treated with ascorbic acid at a dose of 200 mg/kg body weight for 20 minutes before injection of doxorubicin (DOX: 6 mg/kg), mitomycin C (MMC: 0.5 mg/kg) or X-ray (0.5-6 Gy). Mice were then immediately exposed to a 5 T SMF for 24 hours. After exposure to the SMF, bone marrow smears were stained with May-Grünwald Giemsa. The number of micronucleated polychromatic erythrocytes in 1000 polychromatic erythrocytes was counted in each animal under a light microscope. Extracted DNA from bone marrow cells was hydrolyzed by nuclease P1 and alkaline phosphatase, and then did ultrafiltration of the hydrolyzate with Microcon YM-10 filter. 8-OHdG concentration was measured by an ELISA Kit.

2.3 SMF Treatment for tumor bearing mice

A passage of the L1210 mouse leukemia cells used a DBA2 mouse strain. Superconducting magnet was used as a SMFs exposure system. BDF1 mice, transplanted 5x10^6 of L1210 leukemia cells, were co-exposed to bleomycin (5mg/kg/day for 5 days) and 5 T SMFs until they died. An anti tumor effect of co-exposure to both SMF and bleomycin was estimated by the increase of life span (ILS).

ILS = {average of life span in exposure group÷ average of life span in control group)-1} x 100 (%) 

ILS is an index of beneficial effect of the anti tumour drug (written in the Drug Research and Development, National Cancer Institute (USA)). It is said that ISL is significant in the case of 25%.

3. Results

3.1 Mutagenicity of magnetic fields

In Ames test, no statistically significant difference in mutation frequency was observed between exposed and control groups in both TA98 and TA100 strain in every experimental condition.

In yeast mutation assay, exposure to a 5 T static magnetic field resulted in a slight but significant increase in gene conversion frequency in ARG4 locus while reverse mutation in lys1-1 was not altered in mutagenicity assay (Table 1). On the other hand, no mutagenic effect was observed other experimental condition, such as 50 Hz, 40 mT. This mutagenic effect disappeared on exposure to a 2 T static magnetic field and was even lower than the control on exposure to a 1 T static magnetic field. When cells were exposed to a 0.5 T static magnetic field, there was no difference in the frequency of reverse mutation in both ARG4 and lys1. These results suggest that exposure to a strong static magnetic field shows weak mutagenicity and its threshold would be above 2 T in this tester strain.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>lys+ mutants/10^7 survivor (point mutation)</th>
<th>ARG+ mutants/10^4 survivor (gene conversion/recombination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.7 ± 2.5 a</td>
<td>2.4 ± 0.7</td>
</tr>
<tr>
<td>Static, 0.5 T</td>
<td>N.D.</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>Static, 1 T</td>
<td>10.1 ± 3.0</td>
<td>1.9 ± 0.7</td>
</tr>
<tr>
<td>Static, 2 T</td>
<td>N.D.</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>Static, 5 T</td>
<td>9.8 ± 3.5</td>
<td>3.3 ± 0.8 *</td>
</tr>
<tr>
<td>50Hz, 30 mT</td>
<td>N.D.</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td>50Hz, 40 mT</td>
<td>9.1 ± 2.0</td>
<td>2.7 ± 1.6</td>
</tr>
<tr>
<td>UVB</td>
<td>146.1 ± 20.8 *</td>
<td>18.4 ± 3.9 *</td>
</tr>
<tr>
<td>18 J/m²</td>
<td>311.3 ± 110.3 *</td>
<td>27.4 ± 4.9 *</td>
</tr>
<tr>
<td>36 J/m²</td>
<td></td>
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</tbody>
</table>

Table 1 Mutation frequency by exposure to static or 50 Hz MF in yeast mutation assay.

*a) Standard division from at least three independent experiments
b) Not Determined
In the MLA, the plate efficiency that is representative index of acute toxicity was not affected by exposure to all experimental conditions. In addition, the mutation frequency at tk allele (tk\(^{+\text{e}}\) to tk\(^{-\text{e}}\)) is almost same between an MF exposed and unexposed cells in all experimental conditions.

In in vivo micronuclei test, only exposure to SMF was investigated. The frequency of micronuclei induced by DOX, MMC or X-ray was increased by co-exposure to 5 T SMF, but these increases were inhibited by pretreatment with ascorbic acid. Moreover, 8-OHdG of bone marrow cells was increased significantly after 24 hours exposure to 5 T SMF. 8-OHdG concentration in DNA by irradiation to X-ray (0.5 and 1 Gy) increased significantly after 24 hours co-exposure to 5 T SMF. These increases of 8-OHdG concentration were suppressed by pretreatment of ascorbic acid (data not shown).

3.2 Effect of co-exposure with bleomycin in L1210 bearing BDF1 mice

Mice died within a month since L1210 cells were injected. The ISL was observed 18.5% and 12.3% by exposure to SMF (5 T) and bleomycin (5mg/kg), respectively.

Anti tumour effect of co-exposure to SMF or gradient SMF and bleomycin to the L1210 cells was shown in Fig. 1. The ISL increased to 34.6% by co-exposure to gradient SMFs and bleomycin compared with bleomycin (12.3%) alone groups. Similarly, ISL increased to 27.9% by co-exposure to SMFs and bleomycin. ILS is more than 25%, and an anti tumour effect is expected.

4. Discussion

In overview of our study, slight mutagenic and co-mutagenic potential were observed in strong static magnetic field above 2 T while lack of mutagenic effect was observed in 50 Hz up to 40 mT, 2 kHz up to 1 mT, 10 kHz up to 1.6 mT and 20 kHz up to 3.9 mT in various mutation assays [3-9]. In static magnetic field, mutation frequency in Drosophila melanogaster bearing mei-41 (human ATM homologue) [3], Saccharomyces cerevisiae and E. coli bearing uvrA with several mutagens [6] and Bulb/c mice [7], was significantly increased by exposure to SMF for 24 to 72 hrs while lack of effect found in SOD deficient E. coli [10] up to 13 T. In case of these assays, tester strains have various sensitivity to mutagens and also have different DNA repair abilities. In fact, we found dose response relationship between magnetic field density and mutagenic effect was different among test systems. These different responses, may be depend on DNA repair ability, would be reasonable if the exposure to strong static magnetic field caused an increase of DNA lesion. Although a strong magnetic field even at 5 T obviously does not have enough energy to modify the covalent bond of DNA directly, indirect effects such as increase of oxidative damage by exposure to a strong SMF that reported by Watanabe et al [11]. It is possible this hypothetic effect relate to increase in the mutation frequency that found in our studies. In our study, we found treatment of vitamin E suppressed mutagenic effect by SMF in Drosophila melanogaster [3] and this result also supports the hypothesis of indirect oxidative damage by exposure to SMF. On the other hand, the extent of mutagenicity of strong static magnetic field was estimated to be extremely small by comparison of other mutagens. For example, weak UV exposure (18 J/m²) is approximately 20 times more effective than strong static magnetic field which are at least 10,000 times stronger than those in the environment and long exposure duration (48 hours) in yeast mutation assay, thus this suggests that the effect of static magnetic field even in a 5 T (100,000 times higher than geo-magnetic field) is sufficiently small.

In study of possible application, 5 T SMF or gradient SMF slightly enhanced the anti tumour effect of bleomycin in preliminary experiment. This result suggests that simultaneous treatment of bleomycin and SMF exposure would be effective to leukemia cells such as L1210.
However, it is necessary to evaluate the SMFs exposure condition to improve this possible cancer therapy because ISL of the co-exposure to gradient SMF and bleomycin group was higher than that of the co-exposure to homogeneous SMF (5 T) and bleomycin. To develop this method, it is necessary to apply not only leukemia cell line but also other tumour cell lines such as melanoma, lung cancer, hepatic cancer and so on. In addition, it is important to examine other anti tumour agents, especially free radical producing agent such as adriamycin, X-ray, etc. to screen more effective combination with exposure to SMF because it is known that mode of action of bleomycin is to produce free radicals. In further study, application of antioxidant reagents for this experiment should be investigated to prove a mechanism of anti tumour effect in this test system. Since SMF have already used for clinical diagnosis such as MRI (magnetic resonance imaging), it will not be difficult to introduce this treatment for cancer in future.

In our experience, slight mutagenic effects of MF were found in several test systems but extent of the effects is small even at 5T. It suggests effects of MFs in practical environment such as railway systems, home appliances, etc is extremely small as health risk. However, such small effect would be effective in case of medicine. Therefore, it will be important to investigate both possible biological effect of EMF in the frequency range that have lack of previous knowledge to assess its health risk, and possible application such as treatment of EMF in medicine impartially.

References


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