Effect of Extremely Low-Frequency(ELF) Magnetic Field on Anticancer Drugs Potency

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Effect of Extremely Low-Frequency (ELF) Magnetic Fields on Anticancer Drugs Potency

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There are some reports about combined effects of electromagnetic fields with known carcinogens, toxic physical or chemical agents. In previous study, we observed that magnetic fields (60 Hz, 50 mT) enhance cytotoxicity of mitomycin C on *E.coli* bacterium. However, it has not been clear about the effective condition of magnetic fields (magnetic flux density, frequency, exposure time etc.) on the potency of chemical agents. In this study, we investigated the effective density and the action mechanisms of magnetic fields on the enhancement of drugs potency. The result suggested that the enhancing effect of mitomycin C potency by exposure to magnetic fields depended on the magnetic flux density. The potency of cisplatin, widely used in clinical cancer chemotherapy, was also enhanced by exposure to 60 Hz, 50 mT magnetic fields. The analysis of the potency of supernatant remaining drugs in the culture medium indicated that the intracellular drug potency was increased and extracellular drug potency was decreased by exposure to magnetic fields. The values of drug potency revealed a significant inverse correlation between intracellular and extracellular cells. These results suggested that magnetic fields (60 Hz, 50 mT) change the permeability of cell membrane and influence the drug intake.

***Index Terms*—Anticancer drug, enhancing effect, Extremely Low-Frequency (ELF) magnetic fields, membrane permeability.**

I. Introduction

EXTREMELY LOW-FREQUENCY (ELF) magnetic fields were classified as '2B; possibly carcinogenic to humans' by the International Agency for Research on Cancer [1]. This classification was mainly based on limited human epidemiological evidence for an association between ELF magnetic fields exposure and childhood leukemia. No carcinogenic effects of ELF magnetic fields have been found in animal studies that have been tested. The differences on the evidence from animal and human studies may be presumed that the foods, drinks, and home environments and so on, life styles of humans in epidemiological studies are various.

Then, there are some reports about combined effects of electromagnetic fields with known carcinogens, toxic physical or chemical agents [2]–[6]. Tofani *et al.* reported that the survival time of the C57BL/6 mouse bearing Lewis Lung carcinoma, treated with cisplatin and exposed to ELF magnetic fields was significantly longer than that of mice treated only with cisplatin or only with magnetic fields. On the other hand, when the mice bearing a B16 melanotic melanoma were exposed to magnetic fields with anticancer drug, cyclophosphamide, the survival curve was almost the same as that of mice treated with cyclophosphamide alone, and no synergic effects of cyclophosphamide and magnetic fields were observed. These reports suggested that the combination of magnetic fields and a certain anticancer drug has a positive effect or at least no negative effect in experimental cancer therapy.

In previous study, we observed that magnetic fields (60 Hz, 50 mT) enhance the potency of mitomycin C on *Escherichia.coli* (*E.coli*) bacterium. Mitomycin C is genotoxic, antimicrobial agent, and used as anticancer drug in clinical cancer chemotherapy.

If magnetic fields enable us to enhance the potency of anticancer drug on target region only, the dosage can be reduced and thus side effects can be suppressed. However, there are few detailed data about the effective condition (magnetic flux density, frequency and exposure time etc.) and the action mechanism, of magnetic fields on the potency of chemical agents. In this study, we investigated the effective density of magnetic fields and the action mechanism on the enhancement of anticancer drug potency.

II. Measurements of Anticancer Drugs Potency by Exposure to ELF Magnetic Fields

A. Magnetic Fields Generator, Anticancer Drugs and Cells

The device used to generate ELF magnetic fields was composed of laminated iron cores encircled by coils carrying current. The magnetic fields (60 Hz) were generated in the experimental area by exciting the coils and can reach a flux density of 50 mT with 1% uniformity. To avoid the influence of heat generated by the coil, the temperature change in the experimental area was controlled by circulating water of 36 degree C (Fig. 1).

E. coli W3110 was from National BioResource Project *E.coli* Strain of National Institute of Genetics Japan.

Mitomycin and cisplatin were purchased from Kyowa Hakko Kirin Co., Ltd. and Maruko Pharmaceutical Co., Ltd.

A part of anticancer drugs used in clinical chemotherapy, such as mitomycin C and cisplatin, act on DNA of all living organisms as mutagen that can induce genetic mutation [7]. These drugs such as mutagen act on all living organisms. In this study, the potency of these drugs on bacterial cells under magnetic fields was measured because our present system of ELF magnetic fields generator cannot supply the necessary CO_2 for cultivation of human cells.

B. Measurements of Anticancer Drugs Potency

The potency of drugs was measured from cell viability by colony assay as follows.

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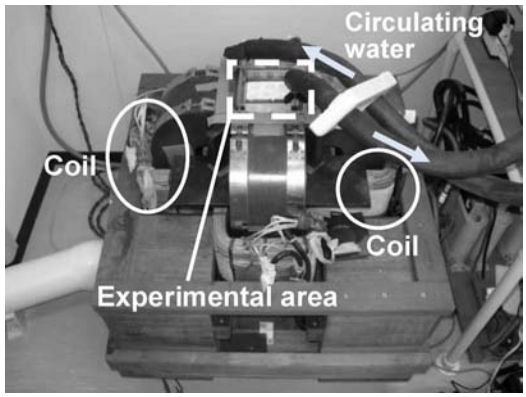

Fig. 1. ELF magnetic fields generator.

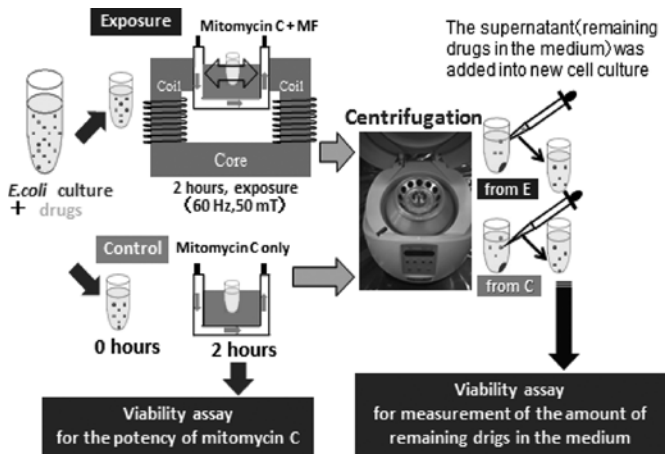

Fig. 2. Protocol for measurement of the potency of intracellular and extracellular drugs.

E.coli cells were grown in LB broth at 36 degree C [8]. *E.coli* culture in logarithmic phase was added drug and divided into two plastic tubes. One tube was placed in magnetic fields exposure area (60 Hz, 5, 30 or 50 mT). Another one was placed in non-exposure area. After reaction, cell culture was plated on LB agar, solid medium. The agar media reduce cell movement and allow cell to propagate into cell clones identified as single colonies. The number of the colonies showed the number of viable cells in sample, since single colony was formed from single bacterial cell. If cell viability is decreased by exposure to magnetic fields, it means that the drug potency is enhanced by magnetic fields. On the other hand, if cell viability is increased, the drug potency is inhibited.

To test whether magnetic fields change the membrane permeability of drugs or not, the potency of the drugs remaining in the medium after exposure to magnetic fields was measured as follows. After non-exposure or exposure to magnetic fields, the *E.coli* culture including drugs was centrifuged (9 000 g $\times$ 10 min), and the *E.coli* cells were precipitated and the supernatants were included the remaining drug in the medium. The supernatants were transferred into new cell culture and the potencies of that were measured by colony assay (Fig. 2).

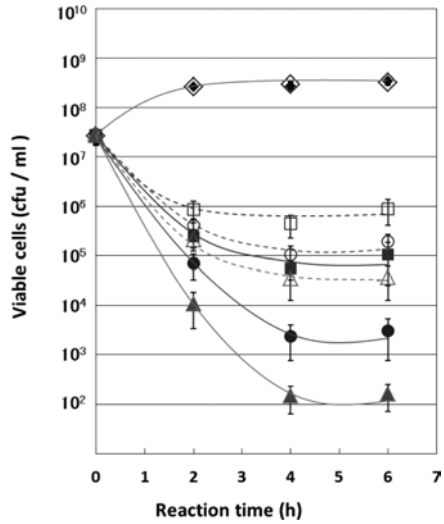

Fig. 3. Effect of 50 mT magnetic fields on the potency of mitomycin C. Cell viability expressed as colony-forming unit/ml. Open symbols, without magnetic fields; filled symbols, with 60 Hz, 50 mT magnetic fields. Mitomycin C (MMC) concentrations are as follows, 0 μg/ml ◇, ◆; 1 μg/ml □, ■; 2 μg/ml ○, ●; 3 μg/ml △, ▲.

III. Effect of ELF Magnetic Fields on Mitomycin C Potency

As shown in Fig. 3, the growth of *E. coli* cells exposed to magnetic fields was the same as that of the non-exposed *E. coli* cells. Therefore, 60 Hz, 50 mT magnetic fields have no influence on the multiplication of *E. coli* cells.

The number of viable cells treated with mitomycin C at 1, 2 and 3 μg/ml decreased from 5×10^7 cfu/ml to 9.0×10^5, 1.9×10^5 and 3.7×10^4 cfu/ml, respectively, in 6 hours. By exposure to 60 Hz, 50 mT magnetic fields, the cells treated with mitomycin C at 1, 2 and 3 μg/ml for 6 hours resulted in decreasing to 1.0×10^5, 3.1×10^3 and 1.6×10^2 cfu/ml, respectively. This result suggested that the viable cells were more decreased by the combination of mitomycin C and magnetic fields than that by MMC only (open symbols to filled symbols in Fig. 3).

Since the potency of mitomycin C depends on the concentration, the relation between mitomycin C concentration and survival rate of the cells at reaction time 6 hours was shown in Fig. 4 as open symbols. Then, the survival rates of the cells treated with mitomycin C and magnetic fields were plotted as filled symbols on the line of survival rate and mitomycin C concentrations. It was indicated that the cytocidal activity of mitomycin C at 1.0, 2.0, and 3.0 μg/ml under 60 Hz, 50 mT magnetic fields has the same as that of 2.3, 4.9 and 6.9 μg/ml of mitomycin C only, respectively (the concentration of filled symbols in Fig. 4). This data suggests that the potency of mitomycin C at each concentration was enhanced about 2.35-fold by exposure to 50 mT magnetic fields for 6 hours. At 5 and 30 mT magnetic fields for 6 hours reaction times, the enhancing rate of the mitomycin C potency was about 1.38 and 1.73-fold, respectively (data not shown).

By contrast, the potency of mitomycin C was not changed when mitomycin C was added in *E.coli* cell culture after exposure to magnetic fields. And, the potency of mitomycin C

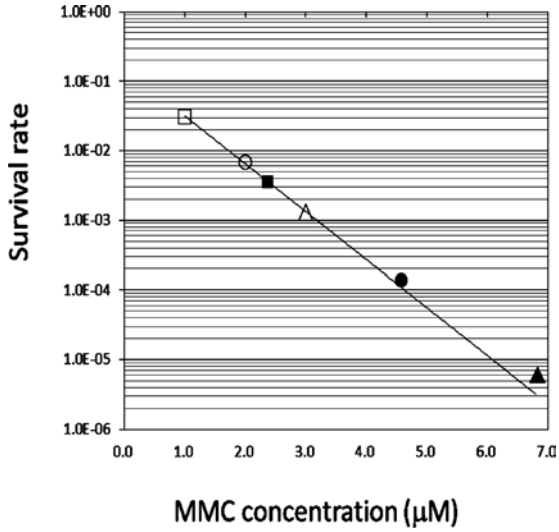

Fig. 4. Relation between mitomycin C concentration and survival rate of the cells at reaction time 6 hours. Open symbols, without magnetic fields; filled symbols, with 60 Hz, 50 mT magnetic fields. Mitomycin C (MMC) concentrations are as follows, 1 μg/ml □, ■; 2 μg/ml ○, ●; 3 μg/ml △, ▲.

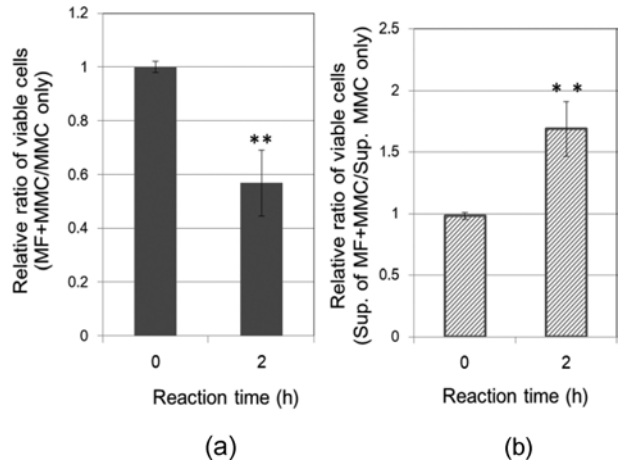

Fig. 5. (a) Comparison of the potency of magnetic field (MF, 60 Hz, 50 mT)+MMC 2 μg/ml and MMC 2 μg/ml only. (b) Comparison of the supernatant, remaining drugs in the medium of MF+MMC 2 μg/ml and MMC 2 μg/ml only. Data are represented as the mean $\pm$SEM from 6 independent experiments, $**P < 0.01$ to MMC only (non-MF Exposure).

was not changed by exposure to magnetic fields of mitomycin C molecules only (data not shown).

These results suggested that magnetic fields can potentiate the cytotoxicity of mitomycin C and that the enhancing effect of mitomycin C under magnetic fields depends on the magnetic flux density.

IV. Effect of ELF Magnetic Fields on Cell Membrane Permeability of Anticancer Drugs

A part of mitomycin C added into the culture medium penetrates the cell membrane, acts on DNA and inhibits DNA replication. Then the extensive DNA damage leads to irreversible injury and results in the cell death. In the above data (Figs. 3 and 4), colony assay measures the rate of the cell death. However, there were the remaining drugs in the culture medium. Therefore, the potency of the drugs remaining in the medium was checked to see whether magnetic fields change the membrane permeability of drugs or not.

In Fig. 5, the values of a vertical axis indicate the relative ratio of the viable cells in the exposure group to the viable cells in control group at each reaction time. When the value is 1.0, it means that the number of viable cell of exposure group is equal to that of control group at that reaction time. Fig. 5(a) shows the comparison between the potency of mitomycin C (2 μg/ml) under magnetic fields (60 Hz, 50 mT) and mitomycin C (2 μg/ml) only. By exposure to magnetic fields for 2 hours, the viable cells were decreased to 0.57, that is, the enhancing rate of mitomycin C potency was increased to 1.90-fold, relative to non-exposure. On the other hand, Fig. 5(b) shows the comparison between the supernatant, remaining drugs in the culture medium of mitomycin C (2 μg/ml) under magnetic fields (60 Hz, 50 mT) and mitomycin C (2 μg/ml) only. By exposure to magnetic fields for 2 hours, the number of viable cells was increased to 1.69-fold, that is, mitomycin C potency of the supernatant was decreased to 0.62-fold, relative to non-exposure.

TABLE I
Effects of 60 Hz, 50 mT Magnetic Fields on Cell Membrane Permeability of Anticancer Drugs

Anticancer drug	Intracellular drug potency ,relative to non-exposure	Extracellular drug potency , relative to non-exposure
Mitomycin C	1.90±0.24** (0.57±0.079)	0.62±0.054** (1.69±0.16)
Cisplatin	1.38±0.11* (0.75±0.069)	0.80±0.052* (1.26±0.074)

The numbers in parentheses show relative ratio of viable cells. Data are represented as the mean ±SEM from 6 independent experiments, *P<0.05, **P<0.01 to non-exposure.

As shown in Table I, intracellular mitomycin C potency was increased to 1.90 and extracellular mitomycin C potency was decreased to 0.62 by exposure to 60 Hz, 50 mT magnetic fields. These values revealed a significant inverse correlation between intracellular and extracellular cells.

In addition, the result of cisplatin, widely used in clinical cancer chemotherapy, was shown in Table I. By combination of magnetic fields and cisplatin (70 μg/ml) for 2 hours, the number of viable cells was decreased to 0.75-fold, relative to non-exposure, cisplatin (70 μg/ml) only. The number of viable cells by cisplatin potency of the supernatant under magnetic fields was increased to 1.26-fold, that is, cisplatin potency of the supernatant was decreased to 0.80-fold, relative to non-exposure. Thus, the intracellular cisplatin potency was increased to 1.38 and extracellular ciaplatin potency was decreased to 0.80 by exposure to 60 Hz, 50 mT magnetic fields. The result of cisplatin shows that the values of drug potency revealed a significant inverse correlation between intracellular and extracellular cells, as well as mitomycin C. These results suggested that ELF magnetic fields change the permeability of cell membrane and influence the drugs intake.

V. Discussion

Several studies have reported that ELF magnetic fields have induced changes in cell membranes [9]–[13]. Ikehara *et al.* observed that 50 Hz magnetic fields (maximum of 41.7 to 43.6 mT) have effects on the peptide linkage and change the secondary structures of helix and sheet in membrane proteins of living Hela cells by using attenuated total reflection infrared spectroscopy [9]. After ELF magnetic exposure for 1 minute, the exposure-caused changes in the secondary structure of protein were nearly recovered within 2 minutes, and the effect on the protein structure was reversible.

Paradisi *et al.* indicated that 50 Hz magnetic fields (2.5 mT, 96 hours) induced significant changes in cell surface structure and physiology by using means of scanning electron microscope and electron paramagnetic resonance spectroscopy [10]. Without altering of cell proliferation, the cell surfaces non-covered by numerous short microvilli were observed by magnetic fields exposure. Similarly, it was revealed that 50 Hz magnetic fields change in plasma membrane morphology and cytoskeletal components in human lymphoblastoid cells Raji by observation of atomic force microscopy in air [10]. Santoro *et al.* observed that 50 Hz magnetic fields (2 mT, 72 hours) decrease in membrane fluidity as detected by Laurdan emission spectromicroscopy and DPH fluorescence polarization. They also showed 50 Hz magnetic fields can interfere with protein phosphorylation by the incorporation of ^{32}P in phosphoprotein and protein electrophoresis analysis (SDS-PAGE) [12]. In addition, Stange *et al.* shown that 50 Hz and 60 Hz magnetic fields increase amino acid uptake into *Vicia faba* L. roots and alter ion movement across the plasma membrane [13]. They suggested that magnetic fields can cause alternation in the permeability of the plasma membrane of plants root cells in vivo.

Those results are consistent with our present results and suggested that ELF magnetic fields induce the change in the cell membrane without effect on cell proliferation.

By the way, tumor cells have got the resistance properties to many unrelated anticancer drugs identified as multidrug-resistant during cancer chemotherapy. One mechanism of drug resistance is thought to be due to the efflux of anticancer drugs caused by P-glycoprotein in cell membrane. Liang *et al.* shows that pulsed magnetic fields enhanced potency of anticancer drug, daunorubicin against multidrug resistant human cell carcinoma subline [14]. They suggested that the pulsed magnetic fields may inhibit the drug efflux-proteins on cell membrane and then modulate daunorubicin potency.

Those reports indicated that exposure to magnetic fields may be a helpful adjunct to chemotherapy. However, further studies are needed to optimize the physical parameters of magnetic fields, type of anticancer drugs and combination effects on human cells.

VI. Conclusion

We investigated the effective density and the action mechanisms, of magnetic fields on the enhancement of drugs potency. The following three points were found in this study. (1) The potencies of drugs such as mitomycin C and cisplatin on *E.coli* cells were enhanced by exposure to 60 Hz magnetic fields. (2) The enhancing effect of the potency of mitomycin C by magnetic fields depended on the magnetic flux density. (3) 60 Hz magnetic fields seem to change the permeability of cell membrane and influence the drug intake. Then, studies on the enhancing effect of anticancer drugs on human culture cells by magnetic fields are now in progress.

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References

[1] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans vol. 80, 2002.

[2] S. Koyama, T. Sakurai, T. Nakahara, and J. Miyakoshi, *Int. J. Radiat. Biol.*, vol. 84, pp. 53–59, 2008.

[3] S. Tofani, D. Barone, M. Berardelli, E. Berno, M. Cintorino, L. foglia, P. Ossola, F. Ronchetto, E. Toso, and M. Eandi, *Pharmacol. Res.*, vol. 48, pp. 83–90, 2003.

[4] G. Ding, H. Yamaguchi, M. Yoshida, and J. Miyakoshi, *Biochem. Biophys. Res. Commun.*, vol. 276, pp. 238–243, 2000.

[5] J. Juutilainen, "Do electromagnetic fields enhance the effects of environmental carcinogens?," *Radiation Protection Dosimetry*, vol. 132, pp. 228–231, 2008.

[6] J. Juutilainen, T. kumlin, and J. Naarala, "Do extremely low frequency magnetic fields enhance the effects of environmental carcinogens? a meta-analysis of experimental studies," *Int. J. Radiat. Biol.*, vol. 82, pp. 1–12, 2006.

[7] M. Kakikawa, M. Oki, H. Tadokoro, S. Nakamura, A. Taketo, and K. I. Kodaira, *Cloning and Nucleotide Sequence of the Major Capsid Proteins of Lactobacillus Bacteriophage ϕ g1e*, *Gene*, vol. 175, pp. 157–165, 1996.

[8] J. Sambrook, E. F. Fritsch, and T. Maniatis, *Molecular Cloning. A Laboratory Manual*, 2nd ed. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1989.

[9] T. Ikehara, H. Yamaguchi, K. Hosokawa, and H. Miyamoto, "K. Aizawa effects of ELF magnetic field on membrane protein structure of living HeLa cells studied by fourier transform infrared spectroscopy," *Bioelectromagn.*, vol. 24, pp. 457–464, 2003.

[10] S. Paradisi, G. Donelli, M. T. Stantini, E. Straface, and W. Malorni, "A 50-Hz magnetic field induces structural and biophysidcal changes in membranes," *Bioelectrimagn.*, vol. 14, pp. 247–255, 1993.

[11] A. Lisi, D. Pozzi, E. Pasquali, S. Rieti, M. Girasole, A. Cricenti, R. Generosi, A. L. Serafino, A. Cohgiu-Castellano, G. Ravanan, L. Giuliani, and S. Grimaldi, "Three dimensional (3D) analysis of the morphological changes induced by 50 Hz magnetic fields exposure on human lymphoblastoid cells (Raji)," *Bioelectromagn.*, vol. 21, pp. 46–51, 2000.

[12] N. Santoro, A. Lisi, D. Pozzi, E. Pasquali, A. Serafini, and S. Grimaldi, "Effect of extremely low frequency (ELF) magnetic field exposure on morphological and biophysical properties of human lymphoid cell line," *Biochimica et Biophysica Acta*, vol. 1357, pp. 281–290, 1997.

[13] B. C. Stange, R. E. Rowland, B. L. Rapley, and J. V. Podd, "ELF magnetic fields increase amino acid uptake into Vicia faba L. roots and alter ion movement across the plasma membrane," *Bioelectromagnetics*, vol. 23, pp. 347–354, 2002.

[14] Y. Liang, C. J. Hannan, Jr., B. K. Chang, and P. V. Schoenlein, "Enhanced potency of daunorubicin against multidrug resistant subline KB-Ch^{R}-8-5-11 by a pulsed magnetic field," *Anticancer Research*, vol. 17, pp. 2083–2088, 1997.