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Original Article

Effect of Static Magnetic Field on the Induction of Micronuclei by Some Mutagens

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Abstract

Objectives: It is important to assess the risk of static magnetic fields (SMFs) on human health, because epidemiological studies have indicated that SMFs play a role in the development of diseases such as leukemia and brain tumor. In our environment, we have numerous chances to be exposed to not only SMFs but also many chemicals containing mutagens. The aim of this study is to investigate the effect of SMFs on the induction of micronuclei induced by some mutagens.

Methods: BALB/c mice were exposed to 4.7 tesla (T) SMF for 24 hr immediately after the injection of carboquone (alkylating agent), colcemid (spindle poison), mitomycin C (cross-linking agent), vincristine (spindle poison), sodium fluoride (a byproduct of aluminum plants under strong SMF) or 1-ethyl-1-nitrosourea (brain tumor-, gliomas- and thymic lymphoma-inducing chemical).

Results: The frequency of micronuclei induced by six mutagens increased after co-exposure to SMF.

Conclusions: An additive/synergistic effect of SMF and chemicals was observed from the results of increased frequency of micronuclei induced by mutagens in mouse bone marrow erythrocytes.

Key words: micronuclei, magnetic fields, mitomycin C, vincristine, co-mutagenic

Introduction

We have many chances to be exposed to not only chemicals, such as food additives, tobacco smoke, medicine, industrial materials, pesticides, and environmental pollutants, but also SMFs from our daily environment, such as those from medical equipment (e.g., magnetic resonance imaging systems), work environments (e.g., in aluminum and magnet production plants) and laboratories (e.g., nuclear magnetic resonance and electron spin resonance systems). Some epidemiological studies have suggested that the incidences of leukemia and brain tumors increase following exposure to SMFs (1). It was also reported that an increase in cancer risk is caused by the additive/synergistic effect of SMFs and chemicals in work environments (1).

There have so far been few studies of the effect of SMFs on the induction of genotoxicity compared with the number of studies of the effect of extremely low frequency magnetic fields. Suzuki et al. reported that strong (higher than 3 T) SMFs have induces micronuclei in mouse bone marrow erythrocytes (2). They considered that one of the mechanisms of the increase in the frequency of micronuclei may be attributable to the stress caused by SMFs. Ikehata et al. showed that exposure to SMFs with a flux density of up to 5 T produced no mutation in *Escherichia coli* WP2 *uvr*A (3, 4). In a co-mutagenicity test, the mutation rate in the group of *Escherichia coli* WP2 *uvr*A treated with six different alkylating agents and simultaneously exposed to 2 T or 5 T SMFs was significantly higher than that for a chemical-alone-exposed group. It is important to elucidate the effect of SMF exposure on the induction of micronuclei in animals treated with chemicals.

The aim of our experiment is to confirm the co-mutagenic effect of SMFs using a micronucleus test.

Materials and Methods

Magnetic field exposure system

The SMF generator used was the superconducting magnet FTNMR JEOL NM-SCM JS-500 (4.7 T, The Japan Electro

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Optical Laboratory). The cylinder bore of the NM-SCM JS-500 measured 200 mm in diameter and 1475 mm in length.

Test chemicals

Carboquone (CQ, Sankyo, CAS: 24279-91-2), colcemid (COL, Hoechst, CAS: 52-28-8), mitomycin C (MMC, Kyowa hakkou, CAS: 50-07-7), vincristine (VCR, Shionogi, CAS: 2068-78-2), sodium fluoride (NaF, Wako, CAS: 7681-49-4), and 1-ethyl-1-nitrosourea (ENU, Sigma, CAS: 759-73-9) were used for the experiments.

Experimental animals

Seven-week-old BALB/c AnNCrlCrlj male mice with body weights ranging from 22–27 g, were obtained from Charles River Japan. The mice were kept in a room that was maintained at a constant temperature and humidity (24±1°C, 42±5%) with a 12 hr light-dark cycle and were given water and CRF-1 diet supplied by Charles River Japan *ad libitum*. Each group subjected to the micronucleus test consisted of more than five male animals. The control groups were maintained under the same conditions as those for the SMF-exposed groups without artificial SMF.

These experiments were performed in accordance with the Guidelines for Animal Experimentation of Jikei University.

Micronucleus test

Dose-response study

The mice were exposed to SMF for 24 hr immediately after the injection of CQ (0.5, 1.0, and 2.0 mg/kg), COL (1.25, 2.5, 5.0, and 7.5 mg/kg), MMC (0.3, 0.5, and 0.7 mg/kg), VCR (0.02, 0.03, and 0.04 mg/kg), NaF (10, 20, and 30 mg/kg) or ENU (9, 18, and 36 mg/kg).

Slide preparation and count

After exposure to SMF, the mice were sacrificed immediately by cervical dislocation. Bone marrow smears prepared as described by Schmid were stained with May Grünwald Giemsa (1/150 M Sörensen's phosphate buffer solution, pH 6.4) (5). The number of micronucleated polychromatic erythrocytes in 1000 polychromatic erythrocytes per animal was counted under a light microscope. The data for micronucleus induction were statistically analyzed by the Kastenbaum-Bowman method (6).

Results

In the experiments, a significant ($p<0.05$) increase in MPCE frequency (COL: 0.84%, CQ and MMC: 0.90%, VCR: 0.92%, NaF: 0.74% and ENU: 0.83%) was observed in the mice that had been exposed only to 4.7 T SMF for 24 hr compared with the non-exposed group, respectively (Figs. 1–6, Table 1).

In the dose-response study, a significant ($p<0.05$) increase in MPCE frequency in the chemical-exposed group was observed 24 hr after treatment with a chemical (COL, CQ, MMC, VCR, NaF or ENU) compared with the non-chemical-exposed group (Figs. 1–6, Table 1). Similarly, a significant ($p<0.05$) increase in MPCE frequency induced by COL, CQ, MMC, VCR, NaF or ENU was observed in the group of mice that were exposed to SMF for 24 hr simultaneously (Figs. 1–6, Table 1).

Table 1 Effect of SMF on the induction of micronuclei by some mutagens

Chemical	Dose (mg/kg)	Frequency of micronuclei (%)	
		Chemical+SMF	Chemical
COL	0	0.84±0.207*	0.10±0.071
	1.25	1.18±0.249*	0.82±0.084
	2.50	1.64±0.321	1.12±0.327
	5.00	2.78±0.356*	1.54±0.313
	7.50	3.63±0.336*	1.92±0.327
CQ	0	0.92±0.133*	0.18±0.044
	0.5	3.02±0.402	1.86±0.230
	1.0	4.24±0.615*	2.92±0.402
	2.0	5.34±0.65*	3.40±0.339
MMC	0	0.90±0.286*	0.20±0.122
	0.3	1.50±0.216	1.06±0.410
	0.5	2.89±0.636	2.04±0.424
	0.7	3.60±0.030*	2.14±0.673
VCR	0	0.92±0.192*	0.20±0.031
	0.02	1.50±0.608*	0.40±0.100
	0.03	1.86±0.385*	1.00±0.141
	0.04	2.86±0.503*	1.70±0.244
NaF	0	0.74±0.150*	0.15±0.080
	10	0.72±0.148*	0.28±0.139
	20	0.83±0.216*	0.33±0.121
	30	1.50±0.200*	0.29±0.126
ENU	0	0.83±0.189*	0.14±0.060
	9	2.24±0.385*	1.44±0.841
	18	2.76±0.397*	1.74±0.623
	36	3.76±0.288*	2.24±0.439

COL, colcemid; CQ, carboquone; MMC, mitomycin C; VCR, vincristine; NaF, sodium fluoride; ENU, 1-ethyl-1-nitrosourea; SMF, static magnetic field.

* $p<0.05$ (The frequency of micronuclei induced by chemicals increased significantly after exposure to SMF).

As for the group exposed to a chemical (COL, CQ, MMC, VCR, NaF or ENU) and SMF simultaneously, MPCE frequency increased significantly ($p<0.05$) higher than that for the chemical-alone-exposed group, and the additive/synergistic effect of SMF and chemicals was observed. The frequencies of micronuclei in the COL (7.5 mg/kg)+SMF, CQ (2.0 mg/kg)+SMF, MMC (0.7 mg/kg)+SMF, VCR (0.03 mg/kg)+SMF, NaF (20 mg/kg)+SMF, and ENU (36 mg/kg)+SMF groups were 1.9, 1.6, 1.7, 1.9, 5.2, and 1.7 times higher than those in the chemical-alone-exposed groups, respectively.

Discussion

We are exposed to not only SMFs but also many chemicals in residential and occupational environments. It is important to analyze the effect of SMFs on mutagenicity induced by mutagens.

Suzuki et al. reported significant time- and dose-dependent increases in micronucleus frequency in mice exposed to 2, 3 or 4.7 T SMFs for 24, 48 or 72 hr. Micronucleus frequency significantly increased following exposure to 4.7 T SMF for all three time periods and to 3 T SMF after 48 or 72 h, whereas

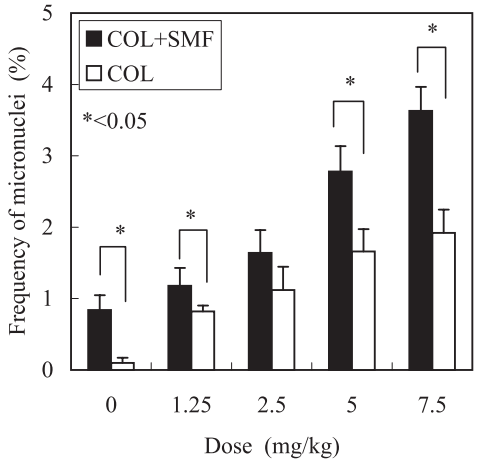

Fig. 1 Effect of static magnetic field (SMF) on the induction of micronuclei by colcemid (COL). The bars in the figure show standard deviations. Each group subjected to the micronucleus test consisted of five male animals.

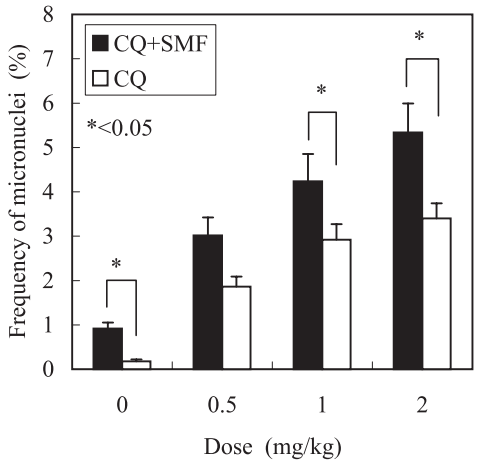

Fig. 2 Effect of static magnetic field (SMF) on the induction of micronuclei by carboquone (CQ). The bars in the figure show standard deviations. Each group subjected to the micronucleus test consisted of five or six male animals.

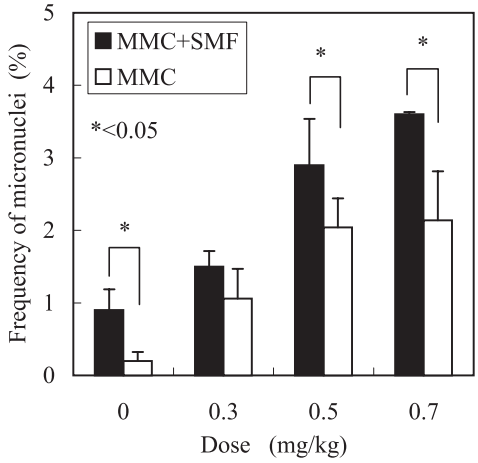

Fig. 3 Effect of static magnetic field (SMF) on the induction of micronuclei by mitomycin C (MMC). The bars in the figure show standard deviations. Each group subjected to the micronucleus test consisted of five or six male animals.

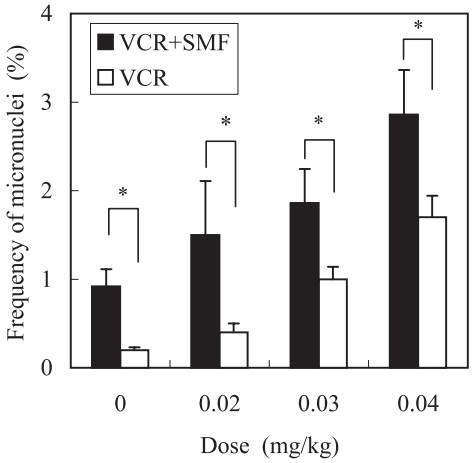

Fig. 4 Effect of static magnetic field (SMF) on the induction of micronuclei by vincristine (VCR). The bars in the figure show standard deviations. Each group subjected to the micronucleus test consisted of three to five male animals.

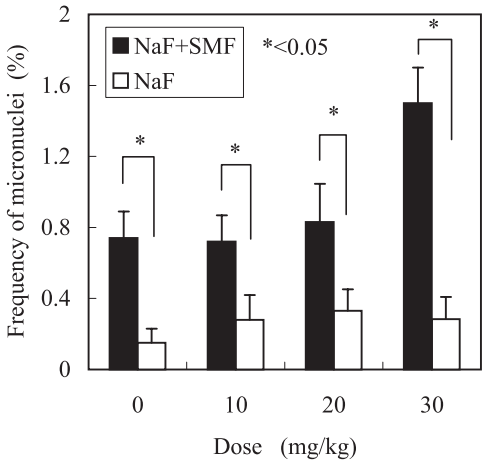

Fig. 5 Effect of static magnetic field (SMF) on the induction of micronuclei by sodium fluoride (NaF). The bars in the figure show standard deviations. Each group subjected to the micronucleus test consisted of three to six male animals.

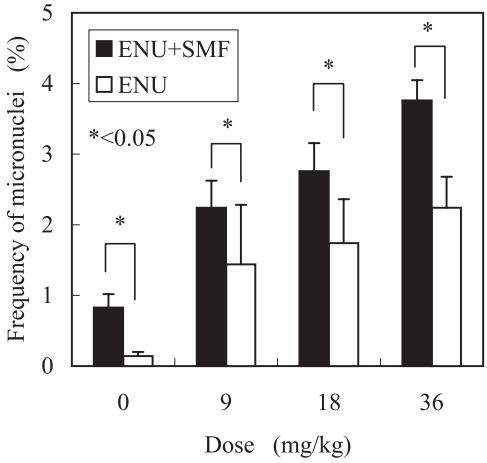

Fig. 6 Effect of static magnetic field (SMF) on the induction of micronuclei by 1-ethyl-1-nitrosourea (ENU). The bars in the figure show standard deviations. Each group subjected to the micronucleus test consisted of five or six male animals.

exposure to 2 T SMF had no significant effect (2). Thus it is possible to detect the mutagenicity of SMFs themselves. For this reason, *in vivo* micronucleus test is considered to be useful for investigating the effect of SMFs on the induction of micronuclei by some mutagens.

We previously reported that the food and drinking water consumption amounts as well as the body weight of mice exposed to 4.7 T SMF were found to decrease after 48 hr exposure compared with those of non-exposed group (7). We also mentioned that mice exposed to strong SMFs of over 1.5 T may feel discomfort because their behavior was depressed. Gollapudi et al. reported that the frequencies of MPCE in the bone marrow of CD-1 male mice after depriving of food and water increased 1.25 times higher at 24 hr, 3.75 times higher at 48 hr and 3.0 times higher at 72 hr than those of intact contrast group (8). Fischman et al. reported that rats subjected to acute behavioral stress show a significantly elevated ratio of sister chromatid exchanges in their bone marrow cells compared with the controls (9). The synthesis of metallothioneins, which act as free radical scavengers or have a protective effect against oxidative stress, may also be induced by SMF (10–12). During the experiment, the amounts of food and drinking water consumed by all mice exposed to SMF decreased (data not shown). The increase in the frequency of MPCE can be attributed to the stress caused by SMF exposure. On the other hand, the production of erythrocytes in bone marrow is enhanced by erythropoietin, a growth factor for erythroblasts (13). The acceleration of erythropoiesis might increase the susceptibility of cells to mutagenic damage because DNA repair is less efficient during DNA synthesis, and spindle formation might be more readily disrupted during rapid cell division (13). We also showed that 4.7 T SMF affects the release of lactate dehydrogenase caused by erythrocyte damage in blood plasma (unpublished data). These results suggest that erythrocyte damage may, therefore, increase the production of renal erythropoietin by a feedback mechanism.

An additive/synergistic effect of SMF and chemicals on the frequency of MPCE induced by all six chemicals (COL, CQ, MMC, VCR, NaF, and ENU) was observed. These results suggest that the treatment of cancer patients with antitumor drugs (CQ, alkylating agent; MMC, cross-linking agent; VCR, spindle poison) together with SMF exposure causes either an additive/synergistic effect on an anticancer therapy or an increase in cancer risks.

Milham suggested that the elevated risk of leukemia among aluminum workers might be associated with exposure to SMFs, resulting from a high direct current used in the electrolytic reduction of alumina to aluminum metal (14). The process used for aluminum production yields fluoride fumes, volatile coal-tar pitch, sulfur oxide, and carbon dioxide. All of these work environmental contaminants must be considered to establish any association between SMF exposure and cancer risk among workers in the aluminum industry. Suzuki reported that sodium fluoride increases the frequency of micronuclei in BALB/c mice (15). The results in this experiment suggest that SMF has an additive/synergistic effect on the induction of micronuclei by sodium fluoride. Under SMF exposure, the frequency of micronuclei by mutagens may increase in mouse bone marrow cells. From these results, the risk of cancer posed by SMFs exposure may, thus, partly be attributable to an additive/synergistic effect.

In an *in vitro* study, Okonogi et al. reported that 4.7 T SMF increased the frequency of micronucleated CHL cells induced by mitomycin C (16). Ikehata et al. reported that the mutation ratio determined by the Ames test in 2- or 5-T SMFs exposed groups was significantly higher than that in non-exposed groups when cells were treated with *N*-ethyl-*N*'-nitro-*N*-nitrosoguanidine, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine, ethylmethanesulfonate, 4-nitroquinoline-*N*-oxide or 2-(2-furyl)-3-(5-nitro-2-furyl) acrylamide (3). Nakahara et al. reported that the exposure of CHO-K1 cells to strong SMF (10 T) has no effects on cell growth, cell cycle distribution, or micronucleus frequency, but it may cause an increase in the micronucleus formation induced by 4 Gy X-rays (17). Takatsuji et al. reported that human lymphocyte exposure to 1.1 T SMF *in vitro* only with co-exposure to alpha or proton particle radiation increases the ratio of chromosomal aberration (18). Koana et al. reported that exposure to 5 T SMF induces somatic mutation in a wing spot test of *Drosophila melanogaster*. They suggested that free radicals are involved in mutagenesis because the mutagenic effect of SMFs disappeared by vitamin E treatment (19). Takashima et al. reported that the dose-response relationship of the mutagenic effect of SMFs (0–14 T) is similar to the lifetime of free radicals in the same test system. They showed that the function of mei-41, a homologue of human ATM (ataxia telangiectasia mutated), may play a role in the induction of mutation by SMFs (20). The data of these reports offer circumstantial evidence that supports the free radical hypothesis for the mutagenic effect of SMFs (19, 20). These findings of *in vitro* and *in vivo* studies suggest that strong SMFs higher than 1 T may increase the frequency of genotoxicity by mutagens or physical factors. Recently, we can be exposed to strong SMFs higher than 1 T during magnetic resonance imaging (MRI) for medical diagnosis. It is necessary to develop safety standards for SMFs intensity and exposure time.

On the other hand, the World Health Organization (WHO) concluded from recent studies that no genotoxic effect of SMFs up to 9 T have been shown, except for one study with repair-deficient bacterial strains. Moreover, the combination of SMFs with other agents, such as genotoxic chemicals, seems to produce synergistic effect, both protective and stimulatory. Further studies should be carried out to clarify these issues. With regard to *in vivo* genotoxicity studies, very few have been carried out such that it is not possible to draw any firm conclusions from the results of these studies (21). It is necessary to carry out an experiment with an accurate exposure condition to confirm the genotoxic effect of SMFs.

Although there are few reports on SMFs, there are several reports concerning the effect of electromagnetic fields (EMFs) on the induction of mutagenicity by chemicals. In EMFs, the co-mutagenic effect of EMFs and X-rays (400 mT at 50 and 60 Hz), mitomycin C (400 mT at 50 Hz) or cisplatin (100 mT at 50 Hz) on genotoxicity was previously observed *in vitro* (22–25). On the other hand, Ansari and Hei reported that concurrent EMF (0.1 mT at 60 Hz) treatment dose not increase either the cytotoxicity or the degree of induction of $CD59^-$ mutants by

graded doses of γ-rays or *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine in A_L cells (26). Mandeville et al. reported that 0.002–2 mT at 60 Hz EMFs have no promoter effect on neurogenic tumors in F344 female rats exposed to ENU transplacentally (27). The intensity of EMF employed in that study is considered to be very low to promote the induction of tumors by ENU. From the published data, EMF stronger than 5 mT may confirm an additive/synergistic effect of mutagens and EMFs on the frequency of genotoxicity or the risks of cancer induced by chemicals. As for the effect of magnetic field on mutagenicity, a difference in magnetic field intensity was observed between SMFs and EMFs. As a result, the effect of EMFs on mutagenicity is, thus, considered to be 200 times stronger than that of SMFs.

In this study, the frequencies of micronuclei in the mouse bone marrow cells induced by mutagens were modified by co-exposure to 4.7 T SMF. Further experiments are needed to elucidate the mechanisms by which SMFs induce cancer.

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