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Induction of micronuclei in mice exposed to static magnetic fields

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The aim of this experiment was to investigate whether static magnetic fields (SMFs) have cytogenetic effects in mouse bone marrow cells. The frequency of micronuclei was significantly increased by exposure of mice to 3.0 T for 48 and 72 h and 4.7 T for 24, 48 and 72 h. The increase in micronucleus frequency was dose dependent at all times. Micronucleus frequency at 4.7 T was higher than at 3.0 T. We consider that the increased numbers of micronuclei may be attributable to a stress reaction caused by SMFs or a direct clastogenic/spindle disturbance effect of SMFs.

Introduction

In recent years static magnetic fields (SMFs) have come to be widely used in research (e.g. nuclear magnetic resonance systems and electron spin resonance), medicine (e.g. magnetic resonance imaging systems) and industry (e.g. in aluminum plants and in magnet production) (WHO, 1987) and thus there is an increased chance of exposure to SMFs. It is important to assess the risk of SMFs to human health, because epidemiological studies have suggested that SMFs may contribute to the induction of leukemia, brain tumors, etc. (Abramovich-Poljakov *et al*., 1979; WHO, 1987). There are many published articles on the genotoxic effects of SMFs or extremely low frequency (ELF) electromagnetic fields. The preponderance of evidence suggests that neither SMFs nor ELF electromagnetic fields demonstrate genotoxic potentials (McCann *et al*., 1993, 1998). On the other hand, there have been no reports on *in vivo* mammalian chromosome effects of SMFs. The *in vivo* micronucleus test has been used as a short-term screening system to detect genotoxic substances and carcinogens (Heddle, 1973; Von Ledebur and Schmid, 1973; Heddle *et al*., 1983). The aim of this experiment was to investigate whether SMFs have cytogenetic effects in mouse bone marrow cells.

Materials and methods

Magnetic field exposure systems

The SMF generators used were a FTNMR JEOL NM-SCM 200/330 superconducting magnet (SCM) (Japan Electro Optical Laboratory, Tokyo, Japan), with a horizontal cylindrical bore of 330 mm diameter and 300 mm long center that generates a homogeneous static field up to 4.7 T, and a Toshiba JS-500 SCM with a horizontal cylindrical bore of 20 cm diameter and 200 mm long center that generates a homogeneous static field up to 3.0 T. These SCM systems were placed in the animal room where the experimental animals were exposed to SMFs.

Experimental animals

Seven-week-old BALB/c AnNCrj male mice of 22–27 g body wt were obtained from Charles River, Japan. Mice housed in polycarbonate cages were kept in a room maintained at constant temperature and humidity (24 ± 1°C, 35 ± 5% for 4.7 T; 23 ± 0.5°C, 50 ± 5% for 2.0 and 3.0 T) on a 12 h light/dark cycle and were given CRF-1 chow (Charles River, Japan) and tap water *ad libitum* during exposure. Each group in the micronucleus test at 4.7 T consisted of five males and the groups at 2.0 and 3.0 T consisted of four males each. The sham exposure control groups were maintained in a cylinder without a SMC in another animal room located near the exposure groups. The intensity of SMF exposure of the sham exposure groups approximated the magnetic field of the Earth. Additional unexposed control groups were fed in an ordinary animal room.

Animal experiments were performed in accordance with the *Animal Experiment Guidelines* of the Jikei University School of Medicine.

Micronucleus test

Mice were exposed to 4.7 T SMFs for 1, 3, 6 and 24 h and samples were collected 24 h after the start of exposure. In other groups mice were exposed to 2.0, 3.0 or 4.7 T SMFs for 24, 48 and 72 h continuously and samples were taken immediately after completion of exposure. An exposure time to SMFs of ⩽72 h was chosen because it is generally accepted that the period of differentiation from stem cell to mature erythrocyte in mice is ~72 h (Filmanowicz and Gurney, 1961). After exposure to SMFs the mice were killed immediately by cervical dislocation and bone marrow cells collected.

Bone marrow smears, prepared as described by Schmid (1976), were stained with May-Grünwald Giemsa (1/150 M Sörensen's phosphate buffer solution, pH 6.4). The incidence of micronucleated polychromatic erythrocytes (MPCE) in 1000 polychromatic erythrocytes (PCE) per animal (coded slides) and the ratio of PCE to normochromatic erythrocytes (P/N) were determined with a light microscope at 1000× magnification. Micronucleus induction data were tested statistically by the Kastenbaum–Bowman method (Kastenbaum and Bowman, 1970). P/N ratio data were analyzed statistically by Student's *t*-test.

Results

In studies involving exposure of mice to 4.7 T SMFs for 1–24 h the frequency of MPCE was significantly increased only after 24 h exposure (with no recovery time), a result reproduced in three experiments (Table I). In each of the experiments MPCE frequency was 2–3 times higher at the 24 h exposure time than in the sham exposure group.

Table II shows the frequency of MPCE in mice exposed to 2.0, 3.0 or 4.7 T SMFs for 24, 48 and 72 h. A significant increase in MPCE ($P < 0.05$) was observed after 48 and 72 h exposure to 3.0 T and at 24, 48 and 72 h to 4.7 T. Exposure to 4.7 T had similar effects in two independent experiments. No significant change in P/N ratio in any exposure group compared with the sham exposure groups was observed.

The frequency of MPCE in the unexposed control groups was almost the same as that in the sham exposure groups (data not shown).

Discussion

The evidence from *in vitro* short-term mutagenicity tests suggests that neither SMFs nor ELF electromagnetic fields have a clearly demonstrated potential to cause genotoxic effects (McCann *et al*., 1993). It was concluded that SMFs up to 1 T do not have a genotoxic effect in microbial systems. The

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Table I. Induction of micronuclei in BALB/c mice exposed to static magnetic fields

	Exposure/recovery time (h)	MPCE frequency (%)	P/N ratio
Experiment 1	0 ± 0	0.40 ± 0.25	1.65 ± 0.60
	1 ± 23	0.46 ± 0.11	1.69 ± 0.31
	3 ± 21	0.48 ± 0.13	1.74 ± 0.20
	6 ± 18	0.50 ± 0.17	1.72 ± 0.11
	24 ± 0	0.87 ± 0.20[a]	1.31 ± 0.29
Experiment 2	0 ± 0	0.24 ± 0.15	1.92 ± 0.64
	1 ± 23	0.45 ± 0.06	1.77 ± 0.24
	3 ± 21	0.42 ± 0.28	1.77 ± 0.40
	6 ± 18	0.44 ± 0.09	1.75 ± 0.27
	24 ± 0	0.82 ± 0.18[a]	1.46 ± 0.38
Experiment 3	0 ± 0	0.24 ± 0.15	1.82 ± 0.75
	1 ± 23	0.44 ± 0.05	1.77 ± 0.25
	3 ± 21	0.43 ± 0.27	1.71 ± 0.43
	6 ± 18	0.56 ± 0.15	1.74 ± 0.25
	24 ± 0	0.82 ± 0.18[a]	1.45 ± 0.01

Mice were exposed to 4.7 T SMFs for 1, 3, 6 and 24 h and samples were taken 24 h from the beginning of exposure in each experiment.
[a]$P < 0.05$.

Table II. Induction of micronuclei in BALB/c mice exposed to 2.0, 3.0 and 4.7 T static magnetic fields (SMFs)

Intensity of SMFs (T)	Exposure time (h)	MPCE frequency (%)	P/N ratio
0	24	0.32 ± 0.13	1.69 ± 0.24
2.0	24	0.31 ± 0.08	1.68 ± 0.28
0	24	0.28 ± 0.13	1.74 ± 0.11
	48	0.30 ± 0.14	1.74 ± 0.24
	72	0.35 ± 0.13	1.83 ± 0.38
3.0	24	0.33 ± 0.22	1.65 ± 0.28
	48	0.88 ± 0.15[a]	1.54 ± 0.36
	72	0.85 ± 0.10[a]	1.77 ± 0.29
0	24	0.21 ± 0.07	1.95 ± 0.21
	48	0.30 ± 0.10	1.94 ± 0.17
	72	0.28 ± 0.12	1.93 ± 0.34
4.7	24	0.87 ± 0.12[a]	1.63 ± 0.05
	48	1.03 ± 0.19[a]	1.65 ± 0.11
	72	1.23 ± 0.17[a]	1.63 ± 0.14
0	24	0.32 ± 0.08	1.93 ± 0.36
	48	0.30 ± 0.21	1.63 ± 0.15
	72	0.28 ± 0.18	1.95 ± 0.15
4.7	24	0.86 ± 0.11[a]	1.82 ± 0.20
	48	0.90 ± 0.19[a]	1.60 ± 0.50
	72	1.10 ± 0.17[a]	1.63 ± 0.15

Mice were exposed to 2.0, 3.0 or 4.7 T SMFs for 24, 48 and 72 h continuously and samples were taken immediately after exposure.
[a]$P < 0.05$.

preponderance of evidence also suggestd that the intensity of SMFs ranging from 0.045 to 1.0 T do not have a genotoxic effect in *Drosophila* or in mammalian cells *in vitro*. We previously observed that an increase in the genotoxic/mutagenic effect of SMFs was not observed below 4.7 T using the *in vitro* micronucleus test with a Chinese hamster cell line (CHL) (Okonogi *et al*., 1996) or below 11.75 T using the Ames test (Shimizu *et al*., 1989). Takatsuji *et al*. (1989), however, reported that human lymphocytes *in vitro* exposed to SMFs at 1.1 T show increased chromosomal aberrations on co-exposure with α-radiation or protons. Koana *et al*. (1995, 1997) reported that SMFs at 5 T caused enhanced somatic recombination in *Drosophila melanogaster* larvae.

On the other hand, there have been no reports of *in vivo* chromosome effects of SMFs. We have demonstrated here, using the micronucleus test, that strong SMFs can have genotoxic effects. We have reported that food and water consumption and body weight of mice exposed to 4.7 T SMFs were reduced after 48 h exposure compared with sham exposure groups (Tsuji *et al.*, 1996). It was also noted that mice exposed to strong SMFs >1.5 T may have felt discomfort, observed as behavior depression. Gollapudi *et al*. (1986) reported that the frequencies of MPCE in the bone marrow of CD-1 male mice were 1.25 times higher at 24 h, 3.75 times at 48 h and 3.0 times at 72 h than a sham exposure group after deprivation of both food and water. Fischman *et al*. (1985) reported that rats subjected to acute behavioral stress had significantly elevated rates of sister chromatid exchange in bone marrow cells compared with the control. Synthesis of metallothioneins, which act as free radical scavengers or participate in protection against oxidative stress (Sato and Bremner, 1993; Satoh *et al*., 1996) and in oxidative stress (Watanabe *et al*., 1997), may also be induced by stress reactions caused by SMFs. The increased number of MPCE may be attributable to the stress caused by SMFs or a direct clastogenic or aneuploidogenic effect of SMFs.

On the other hand, there is a report of induction of micronuclei in male Swiss mice by electric fields (Soheir *et al*., 1989), which, however, differ from SMFs. Mice were exposed to 50 Hz electric fields at intensities of 100, 170, 220 and 290 kV/m for 24 h and then samples were taken at 48, 72 and 96 h after the beginning of exposure. It was reported that MPCE frequency in exposed animals was significantly higher than in controls. A significant dose-dependent increase in MPCE was observed in all experimental groups. However, samples taken 96 h after exposure showed a decrease in the percentage of MPCE, an indication of recovery. We considered that this recovery may be due to the erythroblast–erythrocyte differentiation period. The differentiation period from stem cell to mature erythrocyte is generally thought to be ~72 h. Based on their experimental design, we postulated that 72 h would yield the highest MPCE frequency. Our results show the same frequency of micronuclei until 72 h after exposure to SMFs as after exposure to 50 Hz electric fields. In these *in vivo* studies both SMFs and ELF electromagnetic fields may have induced micronuclei. It is not known, however, if micronuclei induced by SMFs and ELF electromagnetic fields contain fragments or whole chromosomes. Expansion in this direction in future studies would possibly tell us more about the actual mechanisms of micronucleus induction by these factors.

Exposure to strong SMFs may arise from magnetic resonance imaging (MRI), used in clinical diagnosis, and nuclear magnetic resonance (NMR) and electron spin resonance (ESR), used in instrumental analysis. Commercially available MRI, NMR and ESR equipment can generate SMFs of 1.5, 19.0 and 2 T maximum, respectively. Furthermore, we are exposed to SMFs in our daily lives from electrical appliances equipped with a magnet. In this experiment the frequency of micronuclei induced by SMFs in mice was increased time and dose dependently. This suggests that strong SMFs and/or a high integral dose of SMFs may induce micronuclei in humans. It is important to assess the human cancer risk of SMFs and the human exposure level of SMFs in the future.

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