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Teratogenic effects of static magnetic field on mouse fetuses

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Abstract

A short period of exposure of pregnant mice to a strong static magnetic field of 400 mT – 8000 times that of the earth – in a dorso-ventral direction had teratogenic effects on developing fetuses. Fetuses were exposed to the static magnetic field in utero for 60 min on 1 day from 7.5 to 14.5 days of pregnancy. Exposed and control groups consisted of 10 pregnant mice each; thus 160 animals were used in total. Various malformations were observed in 15.1%, 13.4%, 15.8%, 16.7%, 20.8%, 24.3%, 24.4%, and 14.1% of fetuses exposed on days 7.5, 8.5, 9.5, I0.5, 11.5, 12.5, 13.5, and 14.5 of pregnancy, respectively. Types of malformations were polydactylism, abdominal fissure, fused rib, vestigial 13th rib, lumbar rib, brain hernia, and curled tail, while only a low incidence (up to 2.8%) of curled tail was detected in control group. These deformations apparently caused by SMF exposure but the effect did not reflect so-called exposure period specificity.

Keywords: Static magnetic field; Mouse embryo; Malformed fetuses; Polydactylism; Abdominal fissure; Curled tail; Brain hernia

1. Introduction

The development of electrical appliances that use static magnetic and electric fields has increased the possibility of human exposure to these fields and raised concern as to their possible ill-health effects [1–3]. An international seminar by the World Health Organization (WHO) and the International Commission on Non-Ionizing Radiation Protection (ICNIRP) was held in June 1997 on the biological effects and related health hazards of ambient or environmental static magnetic fields (SMFs) [4]. The promoter provided overviews of the scientific contents that were discussed by the scientists who participated in the seminar. Subsequently, Repacholi and Greenebaum [4] formulated a report of this seminar. The working groups of the WHO seminar concluded that exposure to SMFs at levels currently found in the living and working environment, or acute exposure to SMFs at flux densities below 2 T (Tesla, which is a unit to express magnetic flux density), did not have proven adverse human health consequences. However, the reports of biological effects of chronic exposure to SMFs were limited, and for WHO to fully assess any possible human health consequences there is a need to conduct repetitive experiments [4]. Now, in some countries, government committees have issued exposure limits that are intended mainly for magnetic resonance imaging (MRI) in high magnetic fields.

We have conducted research on the biological effects of long-term radio frequency (RF) irradiation of animals. The authors have reported lethal, teratogenic, and delayed hatching effects of 428 MHz RF on developing chick embryos at a power density of 5.5 mW/cm^2 [5,6], where the maximum specific absorption rate (SAR) value was 47 mW/kg and the minimum 3.1 mW/kg. The maximum SAR was below the reported basal metabolic rate in the chick, which means that the exposure level was low enough not to elevate the body temperature to a harmful level. Nevertheless, the average hatchability was 38.0% in exposed eggs and 84.2% in controls [5]. The average number of somites of the early chick embryo (first 48 h) was significantly smaller in the exposed group than in the controls [6]. The developmental delay of irradiated embryos was about 6–16 h in terms of Hamburger–Hamilton staging. It is important to note that there were two cases of duplication abnormalities out of 100 irradiated chick embryos. Unfortunately, the existence of subtle biochemical changes relating to morphogenetic events in embryogenesis stemming from long-term low-level exposure to RF is yet to be fully evaluated. We assume that there may be involvement of magnetic fields caused by RF exposure in any developmental disruption, although this point is difficult to prove.

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In our everyday environment on earth we opportunistically encounter SMF sources. The SMF of earth works at an average flux density around 50 μT. Living creatures have acclimatized to these magnetic field (MF) environments. Only a few species, such as the pigeon, can sense SMFs and utilize them in navigation [7]. Most species appear to have lost this ability, but it is possible that they are still sensitive to much stronger man-made MFs. The strongest electromagnetic fields in the social environment are the SMFs used in MRI techniques [8,9]. MRI systems used for medical diagnosis expose the patient to flux densities as high as 2.5 T [10]. Medical apparatus utilizing static magnetic fields up to 10 T are now being developed [4]. Therefore, the increasing risk of exposure to SMF is becoming a realistic concern for the public.

Few reports have described the occurrence of developmental disruption in cases of exposure of pregnant mammals to SMFs in vivo or exposure of early embryos at the blastocyst stage in vitro [10–14,18–20]. In these reports, MRI has been utilized as the source of the SMF. The researchers placed the animal housed in a cage into the SMF so that the animal could freely move about, randomly crossing the magnetic flux. Under these conditions, no adverse developmental effect of SMFs generated by MRI was found, and a WHO report indicated that acute adverse human health effects would be unlikely to occur at static magnetic flux densities below about 1 T [15]. Although this type of exposure is convenient in experiments where there is relatively long-term continuous exposure to SMFs, we anticipated that adverse effects due to SMF exposure in a constant direction of magnetic flux might have been overlooked. Our conclusion was based on our experience in which we found that exposure to a 428 MHz electromagnetic field resulted in adverse effects in developing chick embryos [5,6]. Regarding invertebrate experiment, Levin and Ernst [16] irradiated SMF at 30 mT for 26 h to the fertilized sea urchin eggs, and obtained decrease in hatching rate from 82% in control to 36% in irradiated group and increase in appearance of exogastrated larva from 2 to 12%. At present, the existence of subtle physiological changes in embryogenesis due to SMF exposure is yet to be fully elucidated. Therefore, we decided to apply an SMF for 1 h on a selected day of pregnancy to pregnant mice fixed on a wooden board between the south and north poles of an electromagnet. A 1 h exposure was selected to avoid the teratogenic effect of heat generated by the electromagnet. Our aim was to explore the effects of SMF exposure on fetal development in mice. We selected a static magnetic flux density of 400 mT—8000 times that of Earth's SMF. It is important that we continue such monitoring of the biological events caused by SMF exposure in animal experiments. Such efforts will improve the current risk assessment of SMF exposure on human health.

2. Materials and methods

2.1. Mice

We used 160 female inbred SS mice aged 60–70 days. All the SS mice at the Department of Veterinary Physiology, Nippon Veterinary and Animal Science University, have been maintained as an inbred strain by sister–brother matings. The animal room was controlled for constant temperature (23–26 °C) and humidity (set at 60%), with a 12 h light–dark cycle (on 6:00 and off 18:00).

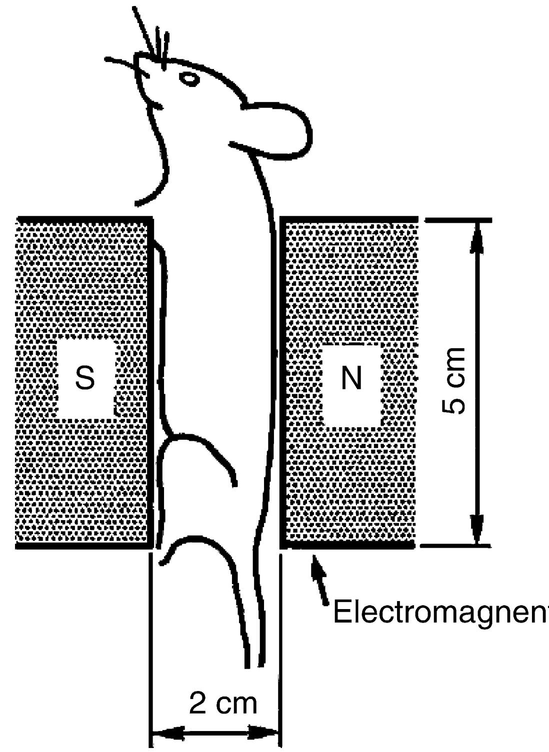

Fig. 1. Schematic representation of static magnetic field (SMF) exposure. The pregnant mouse was fixed on a wooden board, which was inserted into the gap between the north and south poles of an electromagnet.

The animals were fed a commercial diet (CE-2, Japan Clea Co. Ltd., Tokyo) and water ad libitum.

Two or three females were housed overnight in a polycarbonate cage with a male mouse. The next morning female mice were examined for vaginal plugs. When a vaginal plug was detected, the day was designated as day 0.5 of pregnancy.

2.2. Magnetic field exposure system

Fig. 1 shows the SMF irradiation system. The SMF generating apparatus consisted of an electromagnet (model TW-51W, Tokyo Denki Seiki Co. Ltd., Tokyo) and a direct current (DC) power supply (model TMS-1003, Tokyo Denki Seiki Co. Ltd.). The magnetic flux density thus generated was measured by a Gauss meter (model 3251, Yokokawa Electricity Co. Ltd., Tokyo), and the electric current was measured by an ampere meter (model 2011, Yokokawa Electricity Co. Ltd.). A static magnetic field of 400 mT in the electromagnet corresponded to a current of 3 A. The SMF irradiation system was placed in a laboratory room with the ambient temperature kept at 20 °C.

2.3. Detailed SMF exposure design

The operative procedure was undertaken according to the NIH guidelines for the care and use of laboratory animals. Fetuses were exposed to a SMF of 400 mT in utero on one day—day 7.5, 8.5, 9.5, 10.5, 11.5, 12.5, 13.5, or 14.5 of pregnancy—for 60 min. Control mice received sham exposure with the same schedule as that of exposed mice. During the exposure, the mouse was fixed on a wooden board inserted into a gap (2 cm) between the north and south poles of the electromagnet. The mouse was placed ventral to the south pole and thus dorsal to the north pole. Pre- and post-irradiation rectal temperatures (as body-core temperature) were measured with a hand-made digital diode thermometer. Working and sham exposure procedures were carried out alternately on a single exposure per day basis. This alternate-day schedule was designed to ensure complete disappearance of magnetic flux in the electromagnet, which had therefore been used the previous, not the same, day. Exposed and control groups consisted of 10 pregnant mice each in this experiment; thus 160 animals were used in total.

Table 1
Litter size distribution along the day of SMF exposure from GD 7.5 to 14.5 in control and exposed groups

Group	Days of gestation								
	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	Total
Control	8.9 ± 2.0	7.9 ± 1.2	7.6 ± 1.9	7.9 ± 2.5	8.3 ± 2.1	7.7 ± 2.2	9.0 ± 0.6	7.1 ± 1.7	7.9 ± 2.0
Exposed	9.2 ± 1.8	8.2 ± 1.5	7.6 ± 3.1	7.8 ± 2.4	7.7 ± 2.2	7.4 ± 2.6	8.7 ± 1.7	8.5 ± 1.5	8.1 ± 2.3

Exposed and control groups consisted of 10 litters each. Data represent mean and standard deviation. No statistically significant difference was detected between the groups.

2.4. Teratological evaluation

Teratological evaluation was performed on day 18.5. The mouse was sacrificed by an overdose of ether anesthesia, the abdomen opened, and the uterus removed. The numbers of live and dead fetuses and fetal resorption sites were counted. Placentas and live fetuses were removed and weighed. All fetuses were examined for gross malformations and gender. All fetuses were fixed in 95% ethanol for a minimum of 1 week, then skinned and eviscerated. The specimens were double-stained with alcian blue and alizarin red S, and then observed under a stereomicroscope [17]. Cartilage was stained blue and ossified skeleton was stained red.

2.5. Statistical analysis

Student's t-test was used for comparison of average fetal body weights between the same GD groups, with a significance level of $p < 0.01$. Data on skeletal anomalies in control and SMF-exposed groups were tested for statistical significance by chi-squared analysis. Significance levels of $p < 0.01$ or $p < 0.05$ were used.

3. Results

The minimum temperature of the electromagnet before irradiation was 20 °C, which was the same as set for the ambient temperature of the laboratory room. The highest temperature of the electromagnet after irradiation was 31.6 °C. The rectal temperature of the mice was 36–37 °C before irradiation, which became lowered to 30.6 °C in average right after the irradiation and to 30.2 °C after sham irradiation (control), respectively. Post-irradiation rectal temperatures did not differ significantly between irradiation and control groups. Table 1 shows the litter sizes. There were no significant differences in litter size among groups. The average body weights of fetuses in each group are shown in Table 2. There were no significant differences in average body weight between exposed groups and controls. There were also no differences in the sex ratios of fetuses. Fig. 2 shows a case of brain hernia, with the protruding brain covered by thin skin alone. Fusion between the bases of the 11th and 12th ribs was seen in an irradiated fetus (Fig. 3). Polydactylism occurred in the fore- and hind-limbs of the fetus in the SMF-irradiated groups. Formation of extra digits, including duplication of the metacarpus in the fore-limb (Fig. 4), also occurred. In cases of fore-limb polydactylism, the supernumary bone was not connected to the 5th metacarpal. Fig. 5 shows a skeletal specimen of polydactylism in the hind-limb. As in the fore-limb polydactylism, the supernumary bone was not connected to the 5th

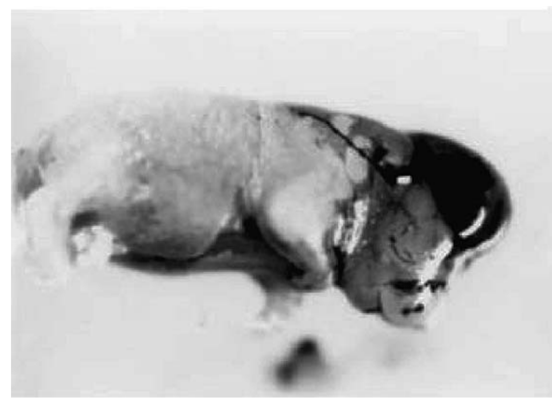

Fig. 2. External appearance of a mouse fetus with brain hernia; this fetus was born to a mother that received SMF irradiation for 1 h on GD 13.5.

Table 2
Body weight of the fetuses on GD 18.5 in SMF exposed and sham exposed groups

	Days of gestation							
	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5
Control								
Male	1.19 ± 0.19 (n = 44)	1.26 ± 0.17 (n = 45)	1.20 ± 0.15 (n = 36)	1.24 ± 0.14 (n = 34)	1.19 ± 0.14 (n = 46)	1.25 ± 0.16 (n = 42)	1.23 ± 0.15 (n = 45)	1.35 ± 0.16 (n = 28)
Female	1.12 ± 0.15 (n = 45)	1.21 ± 0.12 (n = 34)	1.21 ± 0.18 (n = 40)	1.16 ± 0.12 (n = 45)	1.19 ± 0.17 (n = 37)	1.24 ± 0.18 (n = 26)	1.17 ± 0.14 (n = 45)	1.28 ± 0.15 (n = 42)
Exposed								
Male	1.15 ± 0.16 (n = 49)	1.20 ± 0.14 (n = 45)	1.20 ± 0.17 (n = 33)	1.35 ± 0.14 (n = 37)	1.28 ± 0.14 (n = 32)	1.25 ± 0.16 (n = 42)	1.23 ± 0.14 (n = 52)	1.22 ± 0.21 (n = 47)
Female	1.08 ± 0.14 (n = 44)	1.09 ± 0.16 (n = 37)	1.22 ± 0.18 (n = 43)	1.24 ± 0.13 (n = 41)	1.18 ± 0.16 (n = 45)	1.24 ± 0.18 (n = 37)	1.17 ± 0.11 (n = 34)	1.16 ± 0.19 (n = 38)

Data represent mean and standard deviation expressed in g. No statistically significant difference was detected in females or males between the groups.

Table 3
Incidence distribution of total malformations in control and exposed groups according to gestational days of exposure

	Days of gestation							
	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5
Control	2.3% (n = 89)	1.3% (n = 79)	0% (n = 76)	0% (n = 79)	1.2% (n = 83)	1.5% (n = 68)	1.1% (n = 90)	2.8% (n = 71)
Exposed	15.1% (n = 93)[a]	13.4% (n = 82)[a]	15.8% (n = 76)[a]	16.7% (n = 78)[a]	20.8% (n = 77)[a]	24.3% (n = 74)[a]	24.4% (n = 86)[a]	14.1% (n = 85)[b]

N means total number of fetuses at each exposure day in both groups. Malformations included are curled tail, abdominal fissure, rib fusion, lumbar rib and polydactylism. Incidence is expressed as percent occurrence against whole fetuses in a given group.
[a] Significantly different from control at $p < 0.01$ by chi-square method.
[b] Significantly different from control at $p < 0.05$ by chi-square method.

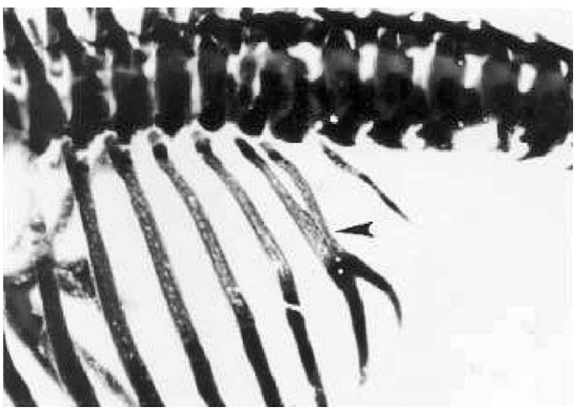

Fig. 3. Lateral view of the dorso-lumbar region of a skeletal specimen of a fetus that received SMF irradiation for 1 h on GD 13.5. Note fusion between 12th and 13th ribs at their bases (arrow head).

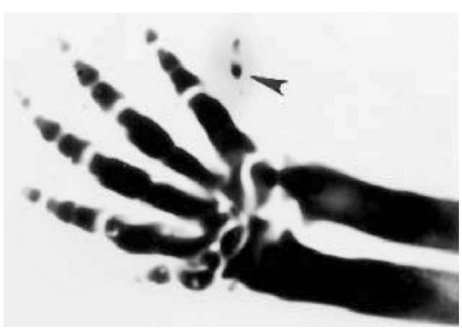

Fig. 4. Skeletal specimen of fore-limb polydactylism in a fetus irradiated with SMF in utero for 1 h on GD 9.5. The extra digit (post-axial position) appears to be floating loose, with no connection to the corresponding metacarpal bone (arrow head).

Fig. 5. Skeletal specimen of hind-limb polydactylism in a fetus irradiated with SMF in utero for 1 h on GD 7.5. The extra digit (post-axial position) seems to be floating loose, with no connection to the corresponding metatarsal bone (arrow head).

Fig. 6. Skeletal specimen of a curled tail in a fetus irradiated with SMF for 1 h on GD 10.5. Note fusion of the coccygeal vertebrae near the bent part of the tail.

metatarsal. Fig. 6 shows a skeletal specimen of curled tail caused by SMF irradiation. There were fusions of the caudal vertebrae in this case.

The incidences of such malformations in each group are shown in Table 3. Malformations were observed in 15.1%, 13.4%, 15.8%, 16.7%, 20.8%, 24.3%, 24.4%, and 14.1% of fetuses exposed on days 7.5, 8.5, 9.5, 10.5, 11.5, 12.5, 13.5, and 14.5 of pregnancy, respectively. Malformations in the control groups consisted solely of curved tail, which occurred in 2.3%, 1.3%, 1.2%, 1.5%, 1.1%, and 2.8% of fetuses sham-exposed on days 7.5, 8.5, 11.5, 12.5, 13.5, or 14.5 of pregnancy, respectively, and there were no anomalies after exposure on days 9.5 or 10.5.

Significant differences were noted in the incidences of malformations in groups exposed on all pregnancy days compared with each corresponding control group ($P < 0.01$ or 0.05, chi-squared method). Table 4 indicates of malformations classified by types and exposure days. Types of malformation included polydactylism, abdominal fissure, fused rib, vestigial 13th rib, lumbar rib, brain hernia, and curled tail. There was no clear correlation between the organogenetic period of the affected structure and the day of exposure. As mentioned above, the incidence of curled tail was significantly greater in the exposed groups on all days of pregnancy on which it occurred in the control group.

4. Discussion

These results demonstrate evidence of the teratogenic potential of exposing restrained pregnant mice for 1 h on a selected day of pregnancy from gestation days (GD) 7.5–14.5 to an SMF with magnetic flux density at 400 mT. Litter size, fetal weight, and fetal sex ratio were not affected by SMF exposure, suggesting that SMF exposure in an intermittent mode might not show lethal or embryotoxic effects. However, the teratogenic effects of SMF were clearly apparent. There were qualitative differences in teratogenesis, as shown by the different types of malformation between exposed and control groups, and there were quantitative differences in the incidence of curled tail between

Table 4
Incidence of malformations classified by types and exposure days

	Days of gestation							
	7.5		8.5		9.5		10.5	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
Brain hernial	0	0	0	0	0	0	0	0
Abdominal fissure	0	2	0	0	0	1	0	0
Polydactylism	0	2	0	1	0	2	0	4
Fused rib	0	0	0	0	0	1	0	0
Trace of 13th rib	0	0	0	0	0	0	0	0
Lumbar rib	0	0	0	0	0	0	0	0
Curled tail	2	11	1	10	0	9	0	10
	11.5		12.5		13.5		14.5	
	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.	Cont.	Exp.
Brain hernial	0	0	0	0	0	1	0	0
Abdominal fissure	0	3	0	0	0	1	0	1
Polydactylism	0	0	0	3	0	3	0	0
Fused rib	0	0	0	0	0	0	0	0
Trace of 13th rib	0	0	0	0	0	1	0	0
Lumbar rib	0	0	0	0	0	2	0	1
Curled tail	1	13	1	15	1	15	2	10

Malformations other than curled tail are not seen in control groups at all days of exposure. Incidence of the curled tail is increased in exposure groups. Thus, malformations seen in the exposure groups are attributable to SMF exposure, Cont.: control; Exp.: exposed.

the exposed and control groups. Differences in the incidence of abnormal development could not be detected in the control group. Therefore, it is apparent that these anomalies occurred through SMF exposure. If restraint had caused such malformations, then we would have expected to detect the same types of malformations in the controls. This was not the case. The development of polydactylism, abdominal fissure, fused rib, vestigial 13th rib, lumbar rib, and brain hernia could be attributed to in utero SMF exposure. The digits, vertebral column, appendicular structure, and central nervous system may be vulnerable to SMF exposure during embryogenesis. Curled tail -another endpoint of SMF targeting - had an increased incidence in the exposed groups: this was also caused by SMF exposure. We speculated that a dosage effect was operating in curled tail formation, but we could not confirm this speculation because we used only one dosage, 400 mT. Oddly enough, both types of malformation did not show the so-called “period specificity” seen with chemical and radiation-induced teratogenesis. That is, the incidence of each type of malformation did not differ among days of treatment: not all the fetuses showed the same malformation after exposure on the same day. We do not know why this occurred, but we are sure that the malformations were induced by SMF exposure. These results give the first clear and definite evidence of teratogenesis in a mammalian in vivo system caused by SMF exposure at 400 mT for 1 h on a selected day of pregnancy. There are two more issues to discuss. One issue is why our work is the first to detect SMF teratogenic potential in mammals. The second is the possible mechanisms of SMF teratogenesis.

With regard to the first issue, it is noteworthy that most of the experiments on SMF teratogenesis have been carried out in MRI environments [10,11,19,20]. Maternal mice were kept in cages and exposed to SMF. The animals could freely move about in the cage, crossing the magnetic flux randomly. This means that the animal was exposed to the magnetic flux in a certain north–south direction at a given moment, and the next moment the moving animal might receive exposure in the reverse direction. Such situations could have had a neutralizing effect on any potential teratogenic effects of SMF.

Exposures of mice to SMF in an MRI environment have been carried out in different countries ant in different years, including BALB/c mice at 0.35 T [19], CRIFA mice during GD7 to 18.5 at 0.7 T [20], murine blastocysts in vitro at 1.5 T [21], ICR mice at 4.7 T [11] and ICR mice for an hour a day from GD7 to 14 at 6.3 T [10]. The negative results have been consistently obtained even at very high magnetic flux density of 6.3 T. In regard to SMF and time-varying irradiation (50 Hz), no adverse effects have been reported [18]. Reproducible negative results have been confirmed in review papers [1–4,15]. The WHO suggests that acute adverse human health effects are unlikely to occur at static magnetic flux densities below about 1 T [15].

Our exposure conditions of 400 mT were closest to those of Henrichs et al. [19] and Tablado et al. [20], although their exposure periods were much longer than ours. Our condition produced malformations, but their conditions did not. Because stronger exposure did not produce alterations, it is difficult to explain the difference as related to stronger and longer exposure to SMF. As mentioned above, we kept the animals restrained between the north and south poles of the electromagnet during exposure, so that exposure with constant magnetic flux direction was possible. If one would suspect a possibility of effects from stress related to keeping temperature homeostasis, teratogenic potential due to the stress caused by restrain would substantially denied, because kinked tail anomalies alone were detected in the sham irradiated control group at very low frequency. We conclude that this difference in exposure procedure was the only factor that determined whether or not malformations would occur. Even with our exposure conditions under restraint, the pregnant uterus could move in the mother's abdominal cavity, thus causing unstable exposure to the fetus, depending on the intra-uterine position. This might explain why not all the fetuses were affected.

The second point of discussion is the mechanism of SMF teratogenesis, including the issue of why there was no period specificity. One possible cause of SMF-induced teratogenesis is high temperature. Lary et al. confirmed the teratogenic effect of hyperthermia in maternal mice and rats with body temperatures of more than 41.5 °C [22–24]. We have previously examined the teratogenic effects of RF radiation on pregnant mice [25]. Types of malformations found were polydactylism, abdominal fissure, fused rib, vestigial 13th rib, and brain hernia—similar to those seen in the present study. The rectal temperature of the RF-irradiated mother mice rose to an average of 42.4 °C. In the present study, we measured the temperature of the mouse placed between the north and south poles of the electromagnet before and after exposure. No such rise in temperature of the mice was detected in this experiment. Actually, the rectal temperature became lowered by 6 °C after irradiation and sham treatment (control) as compared with the temperature before irradiation, the temperature decrease being due to the ambient temperature of 20 °C. The irradiation did not produce high temperature that would be teratogenic. There was no evidence that the low ambient temperature was teratogenic, since only kinked tail anomalies were observed at incidences of 0–2.8% in sham irradiated control groups, which fell within the background incidence (unpublished data). Therefore, the teratogenic effect may not have been caused by high ambient temperature.

Recently, a member of the heat shock protein (hsp) family was found to be induced by RF irradiation or SMF [26]. Barnett reported that hsp mRNA detection was a sensitive assay that was useful in determining whether or not developmental processes were affected by heat [1]. At the moment, we have no solid evidence, but at least one member of the hsp family may be induced by SMF irradiation and may play an important role in the teratogenic mechanism.

With regard to the lack of period specificity in the abnormal developments caused by SMF, the differences in the formation of polydactylism between SMF and chemical exposure provide an interesting example. 5-Bromodeoxyuridine (BrdU) has been reported to induce polydactylism in mice [27]. The polydactylism in these chemical-treated mice fetuses is manifested as a bifurcation in the middle digit. Polydactylism in the SMF-irradiated mice fetuses was manifested as a separation of the digital bones. With chemical treatment, polydactylism is most frequently induced in mouse fetuses by exposure after GD 10.5. In our study, polydactylism was found in fetuses treated maternally with SMF on GD 7.5, which is too early to cause polydactylism directly affecting the developing digit primordia. It is known that time-specific programmed cell death (PCD) occurs in the mouse limb bud during GD 10.5–12.5 to assure normal development of the digits. In cases of abnormal digit formation induced by chemicals, delay in the disappearance of PCD has been noted [27]. Involvement of fibroblast growth factors (FGF) and homeodomain-containing transcription factors (HOX) has been suggested in such abnormal development [28]. In the case of the polydactylism caused by SMF exposure, early cascade event(s) leading to PCD might be affected, and the unknown alteration(s) might be retained for delayed phenotypical expression. Further examination of changes in the cascade of gene expression during the fetal period will be necessary to clarify the teratogenic effects of SMF exposure.

In general, in the consideration of teratogenesis caused by physical factors such as heat, X-irradiation, and static and alternating MF exposure, it is important to recognize that these physical factors can modify biochemical reactions to induce phenotypical alterations in the developing animals. We do not know at present what kinds of biochemical process might be affected by SMF exposure. Some transcriptional factors contain metals such as Fe, Cu, Zn, Co, and some of these might be affected by SMF. Also, it is possible that certain factors involving epigenetic events might be affected by SMF. Both possibilities will be examined in future.

Our previous RF exposure experiment on early chick embryos at stage 10 revealed that there arose severe patterning abnormalities including duplication of the body axis [6]. The results suggested that the early stage of the development might be sensitive to the electromagnetic field influence on fundamental patterning mechanisms, which were operating at that time. Thus, we speculate that if the mouse embryos at early developmental stages of cleavage, morula or streak elongation were maternally exposed to SMF, we could get much drastic abnormal phenotypes. We are now planning the experiment of SMF exposure on pregnant mice starting from GD 0.5.

In conclusion, our study firstly presents evidence that high-dose SMF at 400 mT for 1 h from a constant direction on a selected day of pregnancy between GD 7.5 and 14.5 caused malformations in mouse fetuses. Manner of the exposure as a fixed direction would be critical to the teratogenic potential, even though the mechanisms involved would have not been elucidated. This suggests that there is a health risk to human prenatal development, which is an opposed view as has been believed: many teratogenic studies done in the free-moving mouse kept in a cage in SMF accompanied by MRI apparatus reported to be negative. We hypothesized that if the exposure were not fixed to a direction, the teratogenic potential would be disappeared or not emerged. That is why we conclude that this is the first to recognize a possible teratogenic risk in developing mammals caused by a fixed direction exposure to SMF at 400 mT (higher than the earth MF but lower than MRI associated MF) for a short period. Further research on whether SMF exposure gives rise to adverse human health effects is needed to assess the reality of the risk. It is also necessary to carry out long-term toxicology experiments in animals for hazard identification and risk assessment to human health from chronic SMF exposure. Also, further clarifications would be necessary for the mechanisms of teratogenesis caused by SMF exposure including molecular basis of the teratogenesis.

Acknowledgment

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