J. Korea Asso. Radiat. Prot.
Vol. 31 No. 2 : 91~96 (2006)

▣ 論 文 ▣

The Effects of MRI on Mouse Embryos During Fetal Stage

Takashi Nakamura* · Myung-Sun Ryu*,† · Takahiko Sakazaki* · Yuka Itokawa* · ToshihiroMaenaka* · Takashi Masubuchi* · Hiroyuki Sekimoto* · Masayuki Kanehara* · Young-nam Kang*,‡ and Yeun-Hwa Gu*

*Graduate School of Health Science, Suzuka University of Medical Science
† Department of Radiology, Faculty of Medicine, Ulsan University (Asan Medical Center)
‡ Department of Radiation Oncology, The Catholic University of Korea

(2006년 4월 11일 접수, 2006년 6월 1일 채택)

Abstract - The effects of Magnetic resonance imaging (MRI) on mouse embryos at the early stage of organogenesis were investigated. Pregnant ICR mice were exposed on day 8 of gestation to MRI at 0.5 T for 0.5 hour to 3 hours. The mortality rates of embryos or fetuses, the incidence of external malformations, fetal body weight and sex ratio were observed at day 18 of gestation. A significant increase in embryonic mortality was observed after exposure to either 0.5 T MRI for 0.5 hour or 2 hours. However, the exposure to MRI for 1 hour or 3 hours did not induce any significant increase in embryonic mortality when compared with control. External malformations such as exencephaly, cleft palate and anomalies of tail were observed in all experimental groups exposed to each MRI. A statistically significant increase of external malformations was observed in all groups treated with 0.5 T MRI for 0.5 hour and 3 hours. The incidence of external malformations in the mice group exposed to 0.5 T MRI for 0.5-hour was found to be higher than those of mice group exposed to 0.5 T MRI for 2 hours. The effects of MRI on the external malformations might not to be dose-dependent. There was no statistically significant difference in fetal body weight and sex ratio among each MRI exposure groups.

Key words : Embryonic effects, Organogenesis, Malformation, Magnetic Resonance Imaging (MRI)

INTRODUCTION

Embryos and fetuses are more sensitive to the various environmental agents than children or adults[1,2]. Prenatal development can be divided into three stages: preimplantation, organogenesis and the fetal stages[3]. Each stage corresponds to 0 to 4.5 days, 4.5 to 13.5 days, and 13.5 days of gestation to birth in mice, respectively. Biological effects such as intrauterine death and malformation are closely connected with the stages of prenatal exposure to various agents[4,5]. There have been many experimental studies on the developmental effects of ionizing radiation in mice embryos and fetuses[1,4,5]. The major conclusions of experimental studies were that mouse embryos in organogenesis were more sensitive to malformations and relatively less sensitive to lethal effects.

Several studies on mice irradiated with ionizing radiation[1,6] or exposed to magnetic field at various stages of development did not show any teratogenic effects[7,8]. It has been believed in the medical field that there are no effects on the fetus for MRI during pregnancy [8].

Okuda et al. did the experiment to determine

the genetic effect of static magnetic fields (SMF) and suggested that; exposure to SMF may not induce any significant level of genetic changes in the DNA level[8]. Miyakoshi et al. examined the effects of long-term and low-density (0 to 9 mT) exposures of cultured cells to extremely low frequency magnetic fields (ELF-MF) on cell growth and c-myc mRNA expression in Chinese hamster ovary (CHO) cells. No significant difference in the growth rate or c-myc expression of the cells was observed by the ELF-MF exposure[9].

Therefore, as for teratogenic or mutagenic effects, it doesn't known whether it is in magnetic fields or radiofrequency (RF) wave.

However, many physical and chemical agents may have impact on embryos and fetuses. In setting of safety standards, it has been assumed that each agent independently affects the body. Thus, at present, the safety criterion for each agent is determined independently. Kusama et al. reported that the effects of radiation and caffeine or predonin on ICR mice during organogenesis were synergistic[10]. The synergistic effects of two or more agents as well as the effect of single agent should be considered. This study was carried out to examine the effects of MRI during early stage of organogenesis, at day 8 of gestation, in mice. The teratogenic and lethal effects at this stage are of serious concern to humans from the viewpoint of environmental safety, since at organogenesis the pregnant woman herself may not to know the status of the pregnancy[11,12]. In this stage, these agents can induce external malformation or other effects within a very short time. Thus, the stage for induction of initial effects on target primordial could be defined as the time of exposure.

MATERIALS AND METHODS

Experimental animals

A closed colony of ICR (Crj: CD-1) mice were purchased from Charles River Japan Inc. and were housed at a temperature of 21-23℃ and a relative humidity of 50 to 70% with a 12-hour light-dark cycle (the light phase; 6:00 and 18:00). The mice were given free access to food (CA-1, CLEA Japan Inc.) and tap water. One or two female mice of 10 to 18 weeks old and one male mouse of the same age range were mated for only three hours from 6:00 a.m. to 9:00 a.m.. The female mice in which vaginal plugs were detected were assumed to have become pregnant at 8:00 a.m.[13,14].

Exposure to magnetic fields

All the pregnant mice used in this study were treated with MRI on day 8 of gestation. The pregnant mice were placed in special cages made of paper for MRI exposure. They were exposed to single whole-body MRI at 0.5 T for 0.5 hour to 3 hours. A 0.5 T supper-conduct magnetic system with 23 MHz radio frequency (RF) belonging to the Suzuka University of Medical Science was used. MRI exposure was taken in cage of diameter 10 cm^2, and a head coil was put in the center of the MRI device.

A total of 91 dams and 1286 live fetuses were observed in this study including 21 dams and 277 live fetuses that served as controls.

Observation of external malformations and other effects

The pregnant mice were sacrificed by cervical dislocation on day 18 of gestation and the total numbers of corpora lutea in the ovaries, implantation sites and live and dead embryos and fetuses were counted. The live fetuses were removed from the uterus and examined for external gross malformations under a dissecting microscope. The body weight and sex of each live fetus were also recorded.

Statistical analysis

In determining teratological effects, it is not appropriate to consider the total number of fetus/embryo in each group as an experimental unit[14]. Because fetal effects is because effect every the litter (pregnant mouse) is important [4]. The litter (pregnant mouse) was taken into account as an experimental unit in the statistical

analysis of the experimental data. Thus, in the per litter analysis, the average fetal response within a litter was calculated. For statistical tests, we used non-parametric methods, which were Kruskal-Wallis tests for comparisons among groups or Wilcoxon tests for comparisons between two groups[15].

RESULTS

Intrauterine death

Prenatal deaths of embryos and fetuses were divided into three categories: pre-implantation, embryonic and fetal deaths.

The pre-implantation death was calculated by subtracting the number of total implants from the number of corpora lutea in each pregnant mouse. Implantation sites, placental remnants and resorption of embryos were identified as post implantation embryonic deaths.

Dead fetuses with recognizable eyelids were counted as fetal deaths. Numbers of dams, dead embryos, dead fetuses, live fetuses, litter size and fetal body weight in each experimental group are shown in Table 1. The pre-implantation mortality of the control mice was 11.44%. Statistically significant differences in pre-implantation mortalities between control group and MRI 0.5 hour to 3 hours experimental group were observed ($p<0.05$). The mortalities of post-implantation embryos in control group were 7.09%. The mortality of embryos in the MRI 0.5-hour group and the 0.5 T MRI 2 hours group, 15.05% and 9.09% was significantly higher than that of the control group ($p<0.05$).

There were no significant differences in the mortality of fetal effect between control group and 0.5 T MRI from 0.5 hour to 3 hours group. The mortality in the fetal stage in 0.5 T MRI 2 hours group was marginally significant ($p=0.07$).

Table 1. Embryonic/fetal death and fetal body weight of ICR mice to MRI treated with 8 dpc during organogenesis period.

Groups (Dose/Time; hour)	No. of Dam	No. of Implantations (Mean±SD)	No. of Embryonic deaths (Mean±SD)	No. of Fetal deaths (Mean±SD)	No. of Live Fetuses (Mean±SD)	Fetal Body Weight(g) (Mean±SD)
Control	21	312 (14.8±2.7)	21 (1.01±0.12)	7 (0.33±0.13)	277 (13.1±1.19)	M: 1.458±0.78 F: 1.404±0.190
0.5h	20	268 (13.4±2.1)	42[a,b] (2.1±0.24)	7 (0.33±0.13)	234[a,b] (11.7±0.81)	M: 1.593±0.14 F: 1.511±0.15
1h	20	240[a,b] (12.0±1.7)	13 (0.6±0.23)	8 (0.40±0.10)	260 (13.0±0.23)	M: 1.531±0.19 F: 1.494±0.164
2h	20	238 (11.9±2.5)	20 (1.0±0.17)	13 (0.65±0.12)	258[a,b] (12.9±0.78)	M: 1.537±0.17 F: 1.472±0.172
3h	20	237[a,b] (11.8±2.5)	5 (1.25±0.11)	3 (0.15±0.04)	257 (12.8±0.56)	M: 1.590±0.13 F: 1.513±0.15

M : Male, F : Female

a : significantly different from control group $p<0.05$ by Wilcoxon tests.

b : significantly different from control group $p<0.05$ by Kruskal-wallis tests.

The implantation, embryonic death and fetal body weight have significantly differences between each treatment groups and control group by Wilcoxon and Kruskal-wallis tests.

We used the t-test for statistical analysis of the fetal body weight to the between each treatment groups and significantly different from control group.

We used JUMP statistics software, and 1 to 1 used Wilcoxon tests and multi groups used Kruskal-wallis tests. We input % per a Dom's for Litter effects.

Table 2. Numbers of fetuses bearing external malformations in mice irradiated at 8 dpc in MRI.

Types of malformation	Control	MRI 0.5	MRI 1.0	MRI 2.0	MRI 3.0
Exencephaly	-	3*	-	1	-
Cleft palate	-	-	1	1	-
Open eye	-	2**	-	1	2**
Anomalies of tail	-	-	2**	-	2**
Total number of malformations(Mean)	0	5(0.25)	3(0.15)	3(0.15)	4(0.2)
Total number of dams	21	20	20	20	20
Incidence of malformations (%±SD)	(0)	(2.14±0.14)[a]	(1.15±0.09)	(1.16±0.07)	(1.55±0.12)[a]
Total number of live fetuses	277	234	260	258	257

* The significant increases in the frequencies of exencephaly were recognized in the group irradiated with MRI of 0.5-hour group ($p< 0.05$) when compared to control by Wilcoxon tests.

** The frequencies of anomalies of tail and open eye in mice irradiated with MRI of 0.5 hour, 1 hour and 3 hours group has significantly differences between each treatment groups and control group by Wilcoxon tests.

a : significantly different from control group $p<0.05$ by Kruskal-wallis tests.
The malformation effect has significantly differences between each treatment groups and control group by Wilcoxon and Kruskal-wallis tests.
We used JUMP statistics software, and 1 to 1 used Wilcoxon tests and multi groups used Kruskal-wallis tests. We input % per a Dom's for Litter effects.

External malformations

External gross malformations observed in fetuses on day 18 of gestation are shown in Table 2. Exencephaly, cleft palate, open eye and abnormal tail were observed in live fetuses treated with 0.5 T MRI from 0.5 hours to 3 hours group. In the group treated with 0.5 T MRI 2 hours group, exencephaly, open eye and cleft palate were observed. In the control group, these malformations were not observed. The frequencies of external malformations in 0.5 T MRI 0.5-hour groups were significantly higher than that of the control group. The frequencies of exencephaly, open eye and anomalies of tail in mice group exposed to 0.5 T MRI 0.5, 1, 3 hours were significantly higher as compared with that of mice irradiated with control group ($p< 0.05$ and $p<0.01$ respectively).

Fetal body weight

The fetal body weights in each group are shown in Table 1. The body weights of female and male control fetuses on day 18 of gestation were 1.458g and 1.404 g, respectively. There was no significant difference in fetal body weights among the groups except for female fetuses irradiated with 0.5 T MRI 0.5, 1, 2 and 3 hours group. There was no statistically significant difference in sex ratio in all-experimental groups.

DISCUSSION

In this study, we have found an increase of embryonic death in mice exposed to 0.5 T MRI for 0.5 hour. Heinrichs et al. did not observe an increase of embryonic death by MRI (static field, 0.35 T) in BALB/c mice[16]. But, Murakami (ICRmice, 6.3 T) and Mevissen et al. (Wistar rats, 30 mT) reported embryonic or fetal death were increased by MRI irradiation[7,17], and Zimmermann and Hentschel [18] (4 T) and Nishikawa (ICR mice, 5-16

Gauss) reported a non-significant increase of embryonic death[19]. The results on the lethal effects of MRI exposure at early stage of organogenesis have been inconsistent until now. Many studies reported that radiation at dose of more than 0.5 Gy induced specific types of external malformations corresponding with the stage of organogenesis[5,20]. However, the teratogenic effects of MRI on embryos/fetus in the experimental animals have not been clear. In our study, Exencephaly, cleft palate, open eye and abnormal tail were observed in ICR mice exposed to 0.5 T MRI for 0.5 hour to 3 hours on day 8 of gestation. Tyndall observed external malformations such as microcephaly in C57BL/6J mice exposed to 1.5 T MRI for 36 minute on day 6.5 of gestation[21]. Heinrichs et al. also recognized the induction of external malformations in BALB/c mice irradiated with 0.35 T MRI[16]. On the other hand, Zimmermann and Hentschel reported no malformations in mice treated with 4 T MRI at day 7, 10 and 13 of gestation[18]. And Konermann and Monig (1 T MRI)[22], Kowalczuk et al. (CD1 mice, 20 mT)[23] and Nishikawa (ICR mice, 5-16 Gauss)[19] did not observe external malformations by MRI exposure in experimental animals.

In this study, we observed that mortality of embryos and incidence of external malformations in 0.5 T MRI 2 hours group were found to be significantly lower than in 0.5 T MRI 0.5-hour groups. These results indicate that the effects of MRI dose response on embryos on day 8 of gestation might be mutually deterministic effect of interaction of embryonic cells.

Tyndall performed a combined exposure with MRI and X-rays at dose of 30 cGy in C57BL/6J and observed no additive or synergistic effects of two agents on external malformations[24]. And, Tyndall observed effects of Magnetic resonance imaging and X-ray on eye development in C57BL/6J mice[25].

The results on the effects of the MRI on external malformations were inconsistent among experiments. The mechanism of teratogenesis of MRI is a hyperthermia by radiofrequency (RF) wave or instability of a cell by a magnetic field. As regards the fetal body weight, Tyndall reported a decrease in C57BL/6J mice exposed to 1.5 T MRI[21]. Carnes and Magin also reported decreases in fetal body weight after exposure to 4.7 T MRI[26]. In our study, the fetal body weight was not changed in each experimental group, in which the litter size was not changed, at the level of significance because of the increased mortality of embryos. Then fetal body weight might not be direct effects of MRI to embryos and fetuses.

REFERENCES

1. Kusama T, Hasegawa Y. Stage differences in developmental disorders in ICR mouse embryos irradiated with gamma rays. Cong. Anom. 1993;33:115-123.
2. Kusama T, Gu YH. Effects of radiation and ultrasound on embryonic development in ICR mice. J. Radiat. Res. 1992;33:67-68.
3. Gu YH, Hasegawa T, Suzuki T. Combined effects of radiation and ultrasound on ICR mice in the preimplantation stage. Ultrasound Med. Biol. 2002;28(6):831-836.
4. Gu YH, Kai M, Kusama T. The embryonic and fetal effects in ICR mice irradiated in the various stages of the preimplantation period. Radiat. Res. 1997;147:735-740.
5. Gu YH. The development effects of Radiation on mice embryos in preimplantation stage. J. Kor. Associ. Radiat. Prote. 1996;21:273-284.
6. ICRP; The recommendations of International Commission on Radiological Protection. Nonstochastic effects of ionizing radiation. Publ.41, Pergamon Press, Oxford. 1984: 1-26.
7. Murakami J, Torii Y, Masuda K. Fetal development of mice following intrauterine exposure to a static magnetic field of 6.3 T. Magn. Reson. Imaging. 1992;10:433-437.
8. Carney AL. Magnetic resonance imaging (MRI): is it safe? Clin Electroencephalogr

XI, 1989;20:12-43.
9. Okuda T, Nishizawa K, Ejima Y, Nakatsugawa S, Ishigaki T, Ishizaki K. The effects of static magnetic fields and X-rays on instability of microsatellite repetitive sequences. J. Radiat. Res. 1998;39:279-287.
10. Miyakoshi J, Ohtsu S, Shibata T, Takebe H. Exposure to magnetic field (5 mT at 60Hz) does not affect cell growth and c-myc gene expression. J. Radiat. Res. 1996;37:185-191.
11. Kusama T, Yoshizawa Y. The synergistic effects of radiation and caffeine on embryonic development in mice. J. Radiat. Res. 1984;25:225-233.
12. Kusama T, Sugiura N, Kai M, Yoshizawa Y. Effects of radiation and caffeine on embryonic development in mice. Radiat. Res. 1989;117:273-281.
13. Streffer C, Molls M. Cultures of preimplantation mouse embryos: A model or radiobiological studies. Adv. Radiat. Biol. 1987;13:169-212.
14. Haseman JK, Hogan MD. Selection of the experimental unit in teratology studies. Teratology 1975;12:165-172.
15. Wilson JG, Fraser FC. Current status of teratology - General principles and mechanisms derived from animal studies. In Handbook of Teratology. General principles and etiology. Plenum Press 1979;1:47-74.
16. Heinrichs WL, Fong P, Flannery M, Heinrichs SC, Crooks LE, Spindle A, Pedersen RA. Midgestational exposure of pregnant BALB/c mice to magnetic resonance imaging conditions. Magn. Reson. Imaging. 1988;6:305-313.
17. Mevissen M, Buntenkotter S, Loscher W. Effects of static and time-arying (50-Hz) magnetic fields on reproduction and fetal development in rats. Teratology 1994;50: 229-237.
18. Zimmermann B, Hentschel D. Effect of a static magnetic field (3.5 T) on the reproductive behavior of mice, on the embryo and fetal development and on selected hematologic parameters. Digitale Bilddiagn 1987;7:155-161.
19. Nishikawa U. Biological effects of pulsing electromagnetic field (PEMFs) on ICR mice. Nippon Seikeigeka Gakkai Zasshi 1987;61: 1413-1428.
20. Streffer C. Distribution of micronuclei among single cells of pre-implantation mouse embryos after X-irradiation invitro. Cell. Tissue. Kinet. 1980;13:135-143.
21. Tyndall DA. MRI effects on craniofacial size and crown-rump length in C57BL/6J mice in 1.5 T fields. Oral Surg. Oral Med. Oral Pathol. 1993;76:655-660.
22. Konermann G, Monig H. Effect of static magnetic fields on the prenatal development of the mouse, Radiology 1986;26:490-497.
23. Kowalczuk CI, Robbins L, Thomas JM, Butland BK. Saunders, Effects of prenatal exposure to 50 Hz magnetic fields on development in mice: I. Implantation rate and fetal development. Bio. Elec. Magnet. 1994;15:349-361.
24. Tyndall DA. MRI effects on the teratogenicity of x-irradiation in the C57BL/6J mouse. Magn. Reson. Imaging 1990;8:423-433.
25. Tyndall DA, Sulik KK. Effects of magnetic resonance imaging on eye development in the C57BL/6J mouse. Teratology 1991;43: 263-275.
26. Carnes KI, Magin RL. Effects of in utero exposure to 4.7 T MR imaging conditions on fetal growth and testicular development in the mouse. Magn. Reson. Imaging 1996;14:263-274.