The Combined Effects of MRI and X-rays on ICR Mouse Embryos during Organogenesis

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The combined effects of X-rays and magnetic resonance imaging (MRI) on mouse embryos at an early stage of organogenesis were investigated. Pregnant ICR mice were irradiated on day 8 of gestation with X-rays at a dose of 1 Gy and/or MRI at 0.5 T for 1 hour. The mortality rates of the embryos or fetuses, the incidence of external malformations, the fetal body weight and the sex ratio were observed at day 18 of gestation. A significant increase in embryonic mortality was observed after exposure to either 1 Gy of X-radiation or 0.5 T MRI. However, the combined X-rays and MRI did not show a statistically significant increase in embryonic mortality compared with the control.

External malformations, such as exencephaly, a cleft palate and anophthalmia, were observed in mice irradiated with X-rays and/or MRI. The incidence of each malformation in all treated groups increased with statistical significance compared with the control mice. The incidence in mice irradiated with both X-rays and MRI was lower than in mice irradiated with only X-rays.

The combined effects of the combination of radiation and MRI on the external malformations might be antagonistic. There were no statistically significant differences in fetal death, fetal body weight and sex ratio among all experimental groups.

INTRODUCTION

Embryos and fetuses are more sensitive to various environmental agents than children or adults1). Prenatal development can be divided into three stages: preimplantation, organogenesis and fetal stages. The stages correspond to 0 to 4.5 days, 4.5 to 13.5 days, and from 13.5

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days of gestation to birth in mice, respectively. Biological effects, such as intrauterine death and malformation, are closely connected with the prenatal development stage of mice exposed to various agents. There have been many experimental animal studies on the effects of ionizing radiation on the stage of organogenesis. The major conclusions of these experimental studies were that mouse embryos in organogenesis were more sensitive to malformations and relatively less sensitive to lethal effects of ionizing radiation.

Several studies on mice irradiated with MRI at various stages of pregnancy did not show any teratogenic effects. It has been believed in the medical field that there are no effects on fetuses for MRI during pregnancy. Miyakoshi et al examined the effects of ELF-MF on cell growth and c-myc mRNA expression in Chinese hamster ovary (CHO) cells, and did not recognize any genetic effects in CHO cells. However, they observed the effects of the enhancement of gene expression in K1 (CHO-K1) cells of ELF-MF at doses of more than 400 mT.

On these bases, it remains unresolved whether teratogenic or mutagenic effects are induced by magnetic fields.

Many physical and chemical agents may have a combined impact on embryos and fetuses in the environment. However, there have been few experimental data of combined effects on the development of embryos and fetuses. Kusama et al reported that the combined effects of radiation and caffeine or predominate on ICR mice during organogenesis were synergistic. The combined effects of two agents as well as the effect of a single agent should be considered. The aim of this study was to examine the combined effects of radiation and MRI during the 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, a pregnant woman, herself, may not yet know about the pregnancy. X-rays or MRI affects target primordial within a very short time. We are thus able to define the effects of the time of exposure. It is therefore expected that the stage for the induction of initial effects on target primordial cell can be defined as the time of exposure.

**MATERIALS AND METHODS**

**Experimental animals**

A closed colony of ICR(Crj:CD-1) mice was purchased from Charles River Japan Inc and rehoused at a temperature of 21–23°C 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 from A.M. 6:00 to A.M. 9:00. The female mice in which vaginal plugs were detected were assumed to have become pregnant at 8:00.

**Irradiation with linac X-rays and MRI**

All of the pregnant mice used in this study were treated with X-rays and/or MRI on day
8 of gestation. The pregnant mice were placed in special cages made of plastic for X-ray exposure or paper for MRI exposure. They were treated with single whole-body X-rays of 1 Gy at a dose rate of 0.2 Gy/min and/or a single whole-body MRI at 0.5 T for 1 hour. A 4 MV linac X-ray source and a 0.5 T supper-conduct magnetic system belonging to the Suzuka University of Medical Science were used. The MRI equipment is a 0.5 T supper-conduct magnetic system with a 23 MHz radio frequency (RF). A head coil was put in the center of a 10 cm² cage and irradiation was preformed. In the case of irradiation with both X-rays and MRI, magnetic exposure was performed immediately after X-ray irradiation. The number of dams and live fetuses observed in this study were 91 and 1072, respectively, which included a total of 21 dams and 267 live fetuses that served as controls.

Observation of external malformation and other effects
The pregnant mice were euthanized by cervical dislocation on day 18 of gestation and the total numbers of corpora lutea in the ovaries, of implantation sites and of 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
For teratological effects, it is not appropriate to consider the total number of fetuses/embryos in each group as an experimental unit. The litter (pregnant mouse) was taken into account as an experimental unit in a statistical analysis of the experimental data. Thus, in a per-litter analysis, the average fetal response within a litter was calculated. For statistical tests, we used non-parametric methods; a Kruskal-Wallis test for a comparison among groups or a Wilcoxon test for comparisons between two groups.

RESULTS
Intrauterine death
Prenatal deaths of embryos and fetuses were divided into three categories: pre-implantation deaths, embryonic deaths and fetal deaths. A 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. The number of dams, dead embryos, fetuses, and live fetuses as well as the frequencies of embryonic and fetal deaths in each experimental group is shown in Table 1. The pre-implantation mortality of the control mice was 7.77%. No statistically significant differences in pre-implantation mortalities among all experimental groups were observed (p = 0.2). The mortalities of post-implantation embryos in the control group were 2.20%. Each mortality of embryos in the 1 Gy X-rays and the 0.5 T MRI, 7.8% and 8.6%, respectively, was significantly higher than that of
Table 1. Dead and live embryos/fetuses in each experimental group.

<table>
<thead>
<tr>
<th>Dose (Groups)</th>
<th>No. of Dams</th>
<th>No. of Preimplantation Deaths (mean ± SD)</th>
<th>No. of Embryonic Deaths (mean ± SD)</th>
<th>No. of Fetal Deaths (mean ± SD)</th>
<th>No. of Live Fetuses (mean ± SD)</th>
<th>Litter Size mean ± SD</th>
<th>Fetal body Weight (g) mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21</td>
<td>23 (7.77 ± 6.8)</td>
<td>6 (2.2 ± 3.6)</td>
<td>0 (0)</td>
<td>267 (98.2 ± 3.2)</td>
<td>13.0 ± 1.76</td>
<td>M: 1.140 ± 0.147 F: 1.107 ± 0.143</td>
</tr>
<tr>
<td>1 Gy</td>
<td>20</td>
<td>20 (8.26 ± 8.8)</td>
<td>20 (7.8 ± 12.8)b</td>
<td>2 (0.6 ± 2.1)</td>
<td>200 (90.6 ± 13.7)b</td>
<td>11.7 ± 3.07b</td>
<td>M: 1.004 ± 0.136 F: 0.947 ± 0.139c</td>
</tr>
<tr>
<td>0.5 T(MRI)</td>
<td>20</td>
<td>27 (9.38 ± 9.3)</td>
<td>29 (8.6 ± 22.3)b</td>
<td>0 (0)</td>
<td>232 (91.0 ± 22.2)b</td>
<td>13.1 ± 3.30</td>
<td>M: 1.180 ± 0.101 F: 1.094 ± 0.110</td>
</tr>
<tr>
<td>1 Gy + 0.5 T</td>
<td>30</td>
<td>28 (6.59 ± 11.8)</td>
<td>23 (5.6 ± 8.0)</td>
<td>1 (0.2 ± 1.21)</td>
<td>373 (94.0 ± 7.9)</td>
<td>13.2 ± 3.49</td>
<td>M: 1.045 ± 0.098 F: 1.009 ± 0.099</td>
</tr>
</tbody>
</table>

a M: Male, F: Female
b Significantly different from control group p < 0.05.
We used Wilcoxon test for preimplantation, embryonic and fetal death to the between each treatment groups and significantly different from control group.
c Significantly different from control group p < 0.01.
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.

The control group (p < 0.05 and p < 0.05).
There were no significant differences in the mortality of embryos between the control group and the combined group treated with both 1 Gy X-rays and 0.5 T MRI. The mortality in the fetal stage in all experimental groups was not significantly different (p = 0.2).

External malformations
External gross malformations observed in fetuses on day 18 of gestation are given in Table 2. Exencephaly, a cleft palate, open eye, anophthalmia and abnormal tail were observed in live fetuses treated with 1 Gy X-rays. Hydrocephaly, anomalies of the tail and open eye were observed in the 0.5 T MRI group. In the group treated with both X-rays and MRI, exencephaly, open eye, anophthalmia and anomalies of the tail were observed. In the control group, these malformations were not observed. The frequencies of all types of external malformations in groups treated with X-rays or MRI were significantly higher than that in the control group. Each frequency of exencephaly, anophthalmia and anomalies of tail in mice irradiated with both X-rays and MRI was significantly lower as compared with that in mice irradiated with X-rays alone (p < 0.001, p < 0.01 and p < 0.05, respectively).

Fetal body weight
The fetal body weight in each group is shown in Table 1. The body weights of female and male control fetuses on day 18 of gestation were 1.140 g and 1.107 g, respectively. There was no significant difference in the fetal body weights among the groups, except for female...
Table 2. Numbers of fetuses with external malformations.

<table>
<thead>
<tr>
<th>Type of malformation</th>
<th>Control</th>
<th>1.0 Gy</th>
<th>0.5 T (MRI)</th>
<th>1.0 Gy + 0.5 T (MRI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exencephaly</td>
<td>0</td>
<td>8(^a)</td>
<td>0</td>
<td>4(^b)</td>
</tr>
<tr>
<td>Cleft palate</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Open eye</td>
<td>0</td>
<td>1</td>
<td>2(^c)</td>
<td>3(^b)</td>
</tr>
<tr>
<td>Anophthalmia</td>
<td>0</td>
<td>5(^b)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Hydrocephaly</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Anomalies of tail</td>
<td>0</td>
<td>7(^a)</td>
<td>3(^b)</td>
<td>2(^c)</td>
</tr>
</tbody>
</table>

Total number of malformations | 0 | 22 | 6 | 10

Incidence of malformations (% ± SD) | (0) | (7.9 ± 11.4) | (2.0 ± 4.5) | (2.0) ± 5.4

Total number of dams | 21 | 20 | 20 | 30

Total number of live fetuses | 267 | 200 | 232 | 373

\(^a\) Significantly different from control group p < 0.001.
\(^b\) Significantly different from control group p < 0.01.
\(^c\) Significantly different from control group p < 0.05.

We used Wilcoxon test for malformation rate to the between each treatment groups and significantly different from control group.

DISCUSSION

In this study, we found an increase of embryonic death in the group irradiated by 0.5 T MRI for 1 hour. Heinrichs et al\(^{17}\) did not observe an increase of embryonic death due to MRI (static field, 0.35 T) in BALB/c mice. However, J. Murakami\(^{8}\) (ICR mice, 6.3 T) and Mevisen\(^{18}\) (Wistar rats, 30 mT) reported that embryonic or fetal death was increased by MRI irradiation, whereas Zimmermann and Hentschel\(^{19}\) (4 T) and Nishikawa\(^{20}\) (ICR mice, 5–16 Gauss) reported a non-significant increase of embryonic death. The previous results on the lethal effects of MRI irradiation at an early stage of organogenesis have been inconsistent.

Many studies have reported that radiation at a dose of more than 0.5 Gy induced specific types of external malformations corresponding to the stage of organogenesis\(^{7,21}\). However, the teratogenic effects of MRI on embryos/fetuses in experimental animals have not been clarified. In our study, hydrocephaly, anomalies of the tail and open eye were observed in ICR mice irradiated with 0.5 T MRI for 1 hour on day 8 of gestation. Tyndall\(^{22}\) observed external malformations, such as microcephaly in C57BL/6J mice exposed to 1.5 T MRI for 36 minutes on day 6.5 of gestation. Heinrichs et al\(^{17}\) also recognized the induction of external malformations in BALB/c mice irradiated with 0.35 T MRI. On the other hand, Zimmermann and Hentschel\(^{19}\) reported no malformations in mice treated with 4 T MRI at days 7, 10 and 13 of
gestation. Similarly, Konermann and Monig\textsuperscript{23)} (1 T MRI), Kowalczyk\textsuperscript{24)} (CD1 mice, 20 mT), and Nishikawa\textsuperscript{20)} (ICR mice, 5–16 Gauss) did not observe external malformations due to MRI irradiation in experimental animals.

In this study, we observed that the mortality of embryos in the group irradiated with both X-rays and MRI was lower than those in the groups irradiated with X-rays or MRI alone, and that the incidence of external malformations in the mice irradiated with both X-rays and MRI was significantly lower than that in the mice irradiated X-rays alone. These results indicate that the combined effects of X-rays and MRI on embryos on day 8 of gestation might be mutually antagonistic.

Based on a report by Miyakoshi the X-ray-induced mutation rate was found to be enhanced by subsequent exposure to 400 mT ELF\textsuperscript{25)}. From another of this paper, the results also indicate that long-term exposure to 5 mT ELF after X-irradiation enhances the X-ray induced mutation rate. These results suggest that exposure to more than 5 mT ELF may promote the X-ray induced mutation rate\textsuperscript{26)}. However, similar to our results, Miyakoshi examined the combined effects of X-rays and ELF\textsuperscript{26)} by using C3H10T1/2 cells. From the result, these cells were exposure to ELF alone at 5, 50, and 400 mT for 24 h or X-irradiated with 3 Gy followed by ELF exposure. No significant difference in the transformation was observed between the sham-exposed control and ELF exposure from 5 to 400 mT\textsuperscript{27)}.

Tyndall\textsuperscript{28)} performed a combined exposure with MRI and X-rays at a dose of 30 cGy in C57BL/6J mice, and observed no additive or synergistic effects on external malformations. Tyndall\textsuperscript{29)} also observed the effects on eye development in C57BL/6J mice treated with MRI and X-ray effects. The results of the combined treatment of two physical agents on external malformations were inconsistent among experiments. Regarding the fetal body weight, Tyndall\textsuperscript{22)} reported a decrease in C57BL/6J mice irradiated with 1.5 T MRI, and Carness and Magin\textsuperscript{30)} also reported decreases in the fetal body weight after exposure to 4.7 T MRI. In our study, the fetal body weight was not decreased by a treatment in each group, except with 1 Gy X-rays, in which the litter size was decreased to 11.7 ± 3.07, at the level of significance because of the increased mortality of embryos. However the fetal body weight may not be directly related to the effects of irradiation on embryos and fetuses.

As for embryonic death, it was different from the 1 Gy and 0.5 T MRI groups in comparison with that the combined group. Also, as for the malformation rate, regarding embryonic death, there was a difference in compared with the 1 Gy group in the combined group.

Therefore, these reasons are thought to be the effect on repair due to a rise in temperature by MRI irradiation after irradiation.

In the future, more studies are needed to define the combined effects on embryos and fetuses for two or more agents.

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REFERENCES