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■ 논 문 ■

The Developmental Effects of Radiation on ICR Mouse Embryos in Preimplantation Stage

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着床前期에 있어서 ICR Mouse의 胎兒에 대한 放射線 個體 Level 影響의 硏究

具 然 和
保健醫療技術硏究企劃評價團

Abstract - Embryos and fetuses are more sensitive to various environmental agents than are adults or children. The biological effects such as intrauterine death and malformation are closely connected with prenatal exposure very various agents. The sensitivity of these embryonic/fetal effects depends on the stage of pregnancy. From the viewpoint of fetal development, embryonic and fetal stages can be divided into three stages : Preimplantation, organogenetic and fetal. Each stage corresponds to 0 to 4.5days, 4.5 to 13.5days, and 13.5days of gestation in mice, respectively. Many studies on the biologcal effects of mice irradiated by γ-rays at various stages during organogenesis and fetal period have been performed. Based on these results, the dose-effect and dose-response relationships in malformations, intrauterine death, or retardation of the physical growth have been practically modeled by the ICRP(International Commission on Radiological Protection) and other international bodies for radiation protection.

Many experimental studies on mice have made it clear that mice embryos in the preimplantation period have a higher sensitivity to radiation for lethal effects than the embryos/fetuses on other prenatal periods. However, no teratogenic effects of radiation at preimplantation stages of mice have been described in many textbooks.

It has been believed that "all or none action results" for radiation of mice during the preimplantation period were applied. The teratogenic and lethal effects during the preimplantation stage are one of the most important problems from the viewpoint of radiological protection, since the preimplantation stage is the period when the pregnancy itself is not noticed by a pregnant woman.

There are many physical or chemical agents which affect embryos/fetuses in the environment. It is assumed that each agents indirectly effects a human. Then, a safety criterion on each agent is determined independently.

The pregnant ICR mice on 2, 48, 72 or 96 hours post-conception (hpc), at which are preimplantation stage of embryos, were irradiated whole body Cesium-gamma radiation at doses of 0.1, 0.25, 0.5, 1.5, and 2.5 Gy with dose rate of 0.2 Gy/min. In the embryos from the fetuses from the mice irradiated at various period in preimplantation, embryonic/fetal mortalities, incidence of external gross malformation, fetal body weight and sex ratio were observed at day 18 of

gestation. The sensitivity of embryonic mortalities in the mice irradiated at the stage of preimplantation were higher than those in the mice irradiated at the stage of organogenesis. And the more sensitive periods of preimplantation stage for embryonic death were 2 and 48 hpc, at which embryos were one cell and 4 to 7 cell stage, respectively. Many types of the external gross malformations such as exencephaly, cleft palate and anophthalmia were observed in the fetuses from the mice irradiated at 2, 72 and 96 hpc. However, no malformations were observed in the mice irradiated at 48 hpc, at which stage the embryos were about 6 cell stage precompacted embryos. So far, it is believed that the embryos on preimplantation stage are not susceptible to teratogens such as radiation and chemical agents. In this study, the sensitivity for external malformations in the fetuses from the mice irradiated at preimplantation were higher than those in the fetuses on stage of organogenesis.

Key words : hours post-conception (hpc), preimplantation, malformation, exencephaly, cleft palate, anophthalmia, organogenesis, Heiligenberger mice, ICR mice, pyknotic cells.

요약 – 着床前期의 胎兒는 放射線을 비롯한 많은 環境要因에 대하여 感受性이 높은 個體임에도 불구하고 특히 이 시기는 姙娠婦가 自覺적으로 임신을 감지할 수 없는 시기이기에 이러한 여러 환경유해요인으로 부터 의도적으로 피할 수가 없다.

그러므로 착상전기의 영향을 충분히 검토한 후에 의료행위를 취할 것이며 이에 대한 防禦대책도 검토할 필요가 있다. 종래 까지 방사선에 대한 태아영향에 관한 많은 연구결과에 의하면 방사선 및 그 외의 유해요인에 대한 착상전기의 영향은 胚死亡(流産)만이 일어나며 奇形은 誘發하지 않는다고 하여 發生學등 여러 교과서에서 기형은 器官形成期만이 局限해서 일어나는 영향이라고 단정되어 왔었다. 그러나 이 연구결과 착상전기에 있어서도 기형이 유발하여 오히려 器官形成期보다도 감수성이 높다는 것이다.

또한 착상전기에서도 기형유발의 시기특이성을 가지며 여러 종류의 기형이 발생한다는 것이 본 연구로부터 밝혀졌다.

실험동물은 ICR Mouse를 사용했다. ICR Mouse는 일반적으로 태아사망 및 기형실험에 널리 사용되는 것이다. 사육조건은 Conventional 한 조건하에서 사육했으며 Mating 方法은 Female 마우스의 발정기(Sexual Excitement period)에 있는 mouse 질(膣)을 육안 적으로 관찰하여 AM. 6 : 00~AM 9 : 00시까지 3시간만 mate시켰다. AM 9 : 00시에 Vaginal Plug를 관찰하여 임신을 확인했다. Plug가 확인된 마우스는 AM 8 : 00시에 수정(Conception)된 것으로 가정하고 이 시점을 임신 0일 0시로 수정 난의 태아연령을 산정했다. 방사선조사는 ^{137}Cs γ-선을 사용하였으며 임신 마우스의 전신조사를 실시하고 조사한 시기는 각 2, 48, 72, 96hpc이며 조사한 방사선 선량 군은 0.1~2.5Gy이다. 태아영향 관찰지표는 태아 연령은 mate일 오전 8 : 00시를 임신 0일 0시로 환산하여 태아연령 18일에 임신마우스를 Cervical vertebral dislocation에 의해 도살했다. 도살후 해부하여 각 임신 마우스별로 관찰했다. 착상 율을 관찰하기 위하여 황체수를 세었고, 태아사망과 생존태아를 구별했다. 자궁내 사망의 분류는 태아사망을 ①preimplantation death ②Embryonic death ③Fetal death로 분류했다. 착상전사망은 수정후 0~4.5日(1세포기~배반포후기 부화까지)까지의 사망으로써 난소의 황체수(배란 수)와 착상태아(생존태아, 착상흔, 태반유잔, 흡수태아, 침연태아의 합계)로 부터 구할 수 있다. Embryonic death는 수정후 4.5日~13日까지의 사망으로써 Implantation sites, Placental remnants, Resorption of fetus로 관찰된 것이다. Fetal death는 수정후 14日~18日까지의 사망으로써 Maceration of fetus로 관찰되는 것이다. 통계학적 분석은 각 Group의 착상 율과 자궁내사망율을 산출할 때에는 각 임신마우스에 따라 발생빈도가 크게 다르기 때문에 통계처리에는 Non parametric 검정인 Kluskal Wallis 검정을 사용하여 분석하였다. 또한 개체 Level 영향인 착상율, 태아사망, 기형의 threshold dose의 산정에 대해서는 SAS-Logistic 검정에 따라 통계분석을 하여 5%(Ld_5, ED_5) 및 10%×2/3점을 threshold dose로 판단했다. 태아체중에 대해서는 parametric검정인 t-test검정에 의하여 분석했다. 그 결과 착상전기에서도 기형이 유발하며 특히 시기에 따라 일어나는 때와 일어나지 않는 때가 있음을 본 연구로부터 밝혀졌다. 또한 착상전기의 영향으로써 유발되는 기형은 여러 종류의 기형이 발생함이 밝혀졌다. 특히 이시기는 착상전사망 및 胚사망은 방사선 선량에 따라 크게 일어나나 태아사망(Fetal death) 및 태아체중은 有意差가 없었다.

INTRODUCTION

There have been many studies of the effects of radiation at various stages during embryonic and fetal development. So far, many experimental animal studies have made it clear that the embryos on preimplantation stage have high sensitivity for lethal effects, but no sensitivity for malformation to radiation and many other environmental deleterious agents[1-3]. Therefore, it has been described in any textbook on developmental effects of embryos/fetuses that induction of malformations is characteristics of the period of organogenesis[4-8].

Recently, streffer et al. reported that Heiligenberger mice had significant high sensitivity of malformations for irradiation during preimplantations stage[9,10]. If this is true, a dogma on the embryonic effects at preimplantation that there is no susceptibility for malformations, is broken. For this reason, we examined external malformations and other developmental effects for irradiation during preimplantation stage in ICR mice, which were frequently used in examinations on malformations[11-18].

We used radiation as the pathogenic agent for embryos/fetuses because an acute external exposure to radiation can directly affect embryos/fetuses, and produce the initial cellular and tissue damages leading to developmental defects during a very short time. Then the sensitive period for induction of developmental defects can be identified as the time at exposure.

MATERIALS AND METHODS

Experimental Animals and Mating Procedure

A closed colony of ICR(Crj : CD-1) mice were purchased from Chales River Japan Inc. They were housed in a room at the temperature of 21-23℃ and the relative humidity of 50 to 70% with 12-hour light-dark cycle (the light phases starting at 6 : 00 and 18 : 00). The mice were given free access to food (CA-1, CLEA Japan Inc.) and to tap water. One or two female mice 10 to 18 weeks old were placed together with one male mouse of the same age range in the same cage for only three hours from 6 : 00 to 9 : 00. The female mice in which vaginal plug were found were assumed to have become pregnant at 8:00, at which time was designated as day 0 of gestation[19,20].

Irradiation with Gamma Rays

The pregnant mice were placed in plastic cages for exposure, and were treated with a single whole body gamma radiation at 0.1 to 2.5 Gy at dose rates of 0.2 Gy/min. A 80.2 TBq Cs-137 radiation source belonging to the University of Tokyo was used. The time of exposure for embryos were 2, 48, 72 or 94 hpc. The total number of irradiated dams observed in this study was 458 and a total of 44 non-irradiated control dams was also prepared, and 561 non-irradiated live fetuses served as controls.

Observation of External Malformation and Other Effects

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

The embryo at 2, 48, 72 and 96 hpc were removed from oviduct of pregnant mice. The removed embryos were suspended in hypotonic 0.8% potassium chloride for 30 to 80 minutes at room temperature. The embryos were centrifuged and fixed in 3 methanol : 1 glacial acetic acid. Slides were prepared by air-drying and stained by hematoxirine-eosine. The total cells numbers, the numbers of mitotic and pyknotic cells of each embryo were counted under microscopy.

Statistical Analysis

The reproductive parameters in each group, that is the implantation rate at uterus, mortalities of embryo/fetus and so on, were analyzed by variation analysis with 95% or 99% confidence limit. The incidence of each external malformation in each group were analyzed by the Wilcoxon and the kruskal Wallis tests with a 95% or 99% confidence limit[21,22].

RESULTS

Intrauterine Death

Prenatal deaths of embryos/fetuses were divided into three categories, which were preimplantation death and embryonic and fetal deaths of post-implantation. Implantation sites, placental remnants and resorption of embryos were identified as the embryonic death. Preimplantation death define as before implantation death, This time that embryo move from oviduct to uterious.

The fetal death were identified as maceration of fetuses[10]. The mortalities of preimplantation, embryonic and fetal stages mice irradiated at various periods are shown in Tables 1 to 4 and Figs. 1 to 3. The implantation rates of the non-irradiated control mice were 97.1%. In the mice irradiated at 2, 48, 72 and 96 hpc, the mortalities of preimplantation stage increased significantly($P<0.001$).

Table 1. Embryonic/fetal death and fetal body weight of ICR mice irradiated at 2hpc during preimplantation period.

Dose (2hpc)	No. of Dam	No of Implantation(%)	No. of Dead Embryos(%)	No. of Dead Fetuses(%)	No. of Live Fetuses(%)	Fetal body Weight(g)
Control	44	607 (97.13)	45 (7.41)	1 (0.16)	561 (92.42)	M : 1.376± 0.107 F : 1.323± 0.096
0.1Gy	30	392 (94.58)	44 (11.22)	0 (0)	348 (88.7)	M : 1.373± 0.096 F : 1.327± 0.088
0.25Gy	20	324 (88.55)	40 (12.34)	2 (0.65)	282 (87.03)	M : 1.289± 0.157 F : 1.253± 0.150
0.5Gy	21	245 (77.14)	67 (27.73)	1 (0.31)	177 (72.24)	M : 1.393± 0.132 F : 1.349± 0.147

Implantation rate : The value was calculated as the following method.

1) The corpus luteum number of each mouse was counted.

2) The implantation number of each mouse corpus luteum was ruled out.

M : Male, F : Female

Table 2. Embryonic/fetal death and fetal body weight of ICR mice irradiated at 48hpc during preimplantation period.

Dose (2hpc)	No. of Dam	No of Implantation(%)	No. of Dead Embryos(%)	No. of Dead Fetuses(%)	No. of Live Fetuses(%)	Fetal body Weight(g)
Control	44	607 (97.18)	45 (7.41)	1 (0.16)	561 (92.42)	M : 1.376± 0.107 F : 1.323± 0.096
0.25Gy	22	297 (93.72)	27 (9.09)	4 (1.34)	266 (89.5)	M : 1.404± 1.113 F : 1.374± 0.154
0.5Gy	21	252 (90.34)	42 (16.66)	0 (0)	210 (83.33)	M : 1.371± 0.101 F : 1.313± 0.085
1.5Gy	20	254 (81.75)	143 (56.29)	1 (0.39)	110 (43.30)	M : 1.408± 0.097 F : 1.322± 0.102

Implantation rate : The value was calculated as the following method.

1) The corpus luteum number of each mouse was counted.

2) The implantation number of each mouse corpus luteum was ruled out.

M : Male, F : Female

Table 3. Embryonic/fetal death and fetal body weight of ICR mice irradiated at 72hpc during preimplantation period.

Dose (72hpc)	No. of Dam	No of Implantation(%)	No. of Dead Embryos(%)	No. of Dead Fetuses(%)	No. of Live Fetuses(%)	Fetal body Weight(g)
Control	44	607 (99.18)	45 (7.14)	1 (0.16)	561 (92.42)	M : 1.376± 0.107 F : 1.323± 0.096
0.1Gy	38	519 (95.67)	47 (9.05)	5 (0.96)	467 (89.98)	M : 1.398± 0.117 F : 1.328± 0.093
0.25Gy	37	459 (91.22)	40 (8.71)	7 (1.52)	412 (89.72)	M : 1.386± 0.117 F : 1.316± 0.099
0.5Gy	40	532 (89.99)	56 (10.52)	8 (1.50)	468 (87.96)	M : 1.351± 0.096 F : 1.305± 0.093
1.5Gy	36	467 (91.24)	106 (22.69)	2 (0.42)	359 (76.87)	M : 1.341± 0.091 F : 1.302± 0.086
2.5Gy	28	369 (90.73)	217 (58.80)	1 (0.27)	151 (40.92)	M : 1.390± 0.150 F : 1.346± 0.133

Implantation rate : The value was calculated as the following method.

1) The corpus luteum number of each mouse was counted.

2) The implantation number of each mouse corpus luteum was ruled out.

M : Male, F : Female

Table 4. Embryonic/fetal death and fetal body weight of ICR mice irradiated at 96hpc during preimplantation period.

Dose (96hpc)	No. of Dam	No of Implantation(%)	No. of Dead Embryos(%)	No. of Dead Fetuses(%)	No. of Live Fetuses(%)	Fetal body Weight(g)
Control	44	607 (99.18)	45 (7.14)	1 (0.16)	561 (92.42)	M : 1.376± 0.107 F : 1.323± 0.096
0.1Gy	20	290 (94.81)	26 (8.96)	1 (0.34)	263 (90.68)	M : 1.390± 0.095 F : 1.333± 0.098
0.25Gy	20	268 (91.51)	26 (9.70)	3 (1.11)	239 (89.17)	M : 1.331± 0.121 F : 1.314± 0.128
0.5Gy	20	251 (90.83)	29 (11.55)	5 (1.99)	217 (86.45)	M : 1.411± 0.144 F : 1.366± 0.226
1.5Gy	21	281 (89.38)	32 (11.70)	1 (0.35)	247 (87.90)	M : 1.241± 0.115 F : 1.199± 0.133
2.5Gy	20	264 (92.10)	60 (22.72)	1 (0.37)	203 (76.89)	M : 1.216± 0.082 F : 1.212± 0.082

Implantation rate : The value was calculated as the following method.

1) The corpus luteum number of each mouse was counted.

2) The implantation number of each mouse corpus luteum was ruled out.

M : Male, F : Female

In the mice irradiated at 2 and 48 hpc, the mortalities of preimplantation were recognized to be the dose-response relationships. However, in mice irradiated at 72 or 96 hpc, a significant dose response relationships between doses and implantation rates were not recognized. Mortality in embryonic stage (embryonic death rate) in control mice was 7.41%. The mortalities of embryonic stage in mice irradiated at various stages of preimplantation increased significantly($P<0.001$). Particularly in mice irradiated at 2, 48 and 72 hpc, mortalities of embryonic stage were recognized to have the strongest dose-response relationship($P<0.001$). For the mortalities at the fetal stage in all irradiated mice, there were no statistically significantly difference among dose groups. Regarding the mortalities of preimplantation and embryonic stages, our data showed that the embryos irradiated at 2hpc were the more sensitive than those irradiated at 48, 72 and 96 hpc.

External Malformations

External malformations observed in fetuses irradiated at 2, 72 and 96 hpc are shown in Tables 5 to 8. Various types of external malformation which were exencephaly, cleft palate, ventral hernia, open eye, anophthalmia, abnormal tail and polymelia, were observed in fetuses irradiated at 2, 72 and 96 hpc. Polymelia, and open eye were also observed in control fetuses, however other external malformations were not observed in control fetuses. On the other hand, in fetuses irradiated at 48 hpc, external malformations except polymelia were not observed, shown in Tables 6. The embryos on 48 hpc were no sensitive for radiation on external malformations however, the embryos on 2, 72 and 96 hours after conception had high sensitivity for radiation on external malformation.

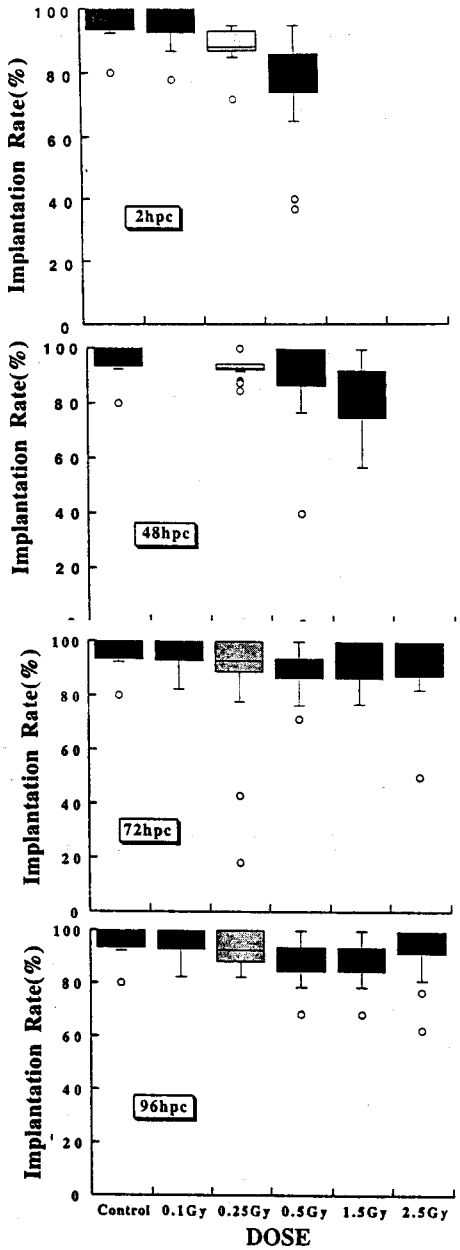

Fig. 1. Implantation rate of ICR mice irradiated at 2, 48, 72 and 96hpc in the preimplantation period. The dose-dependence was detected with statistical significance ($p<0.001$) among all dose groups by Kruskal Wallis test.

Implantation rate : The value was calculated as the following method.

1) The corpus luteum number of each mouse was counted.

2) The implantation number of each mouse corpus luteum was ruled out.

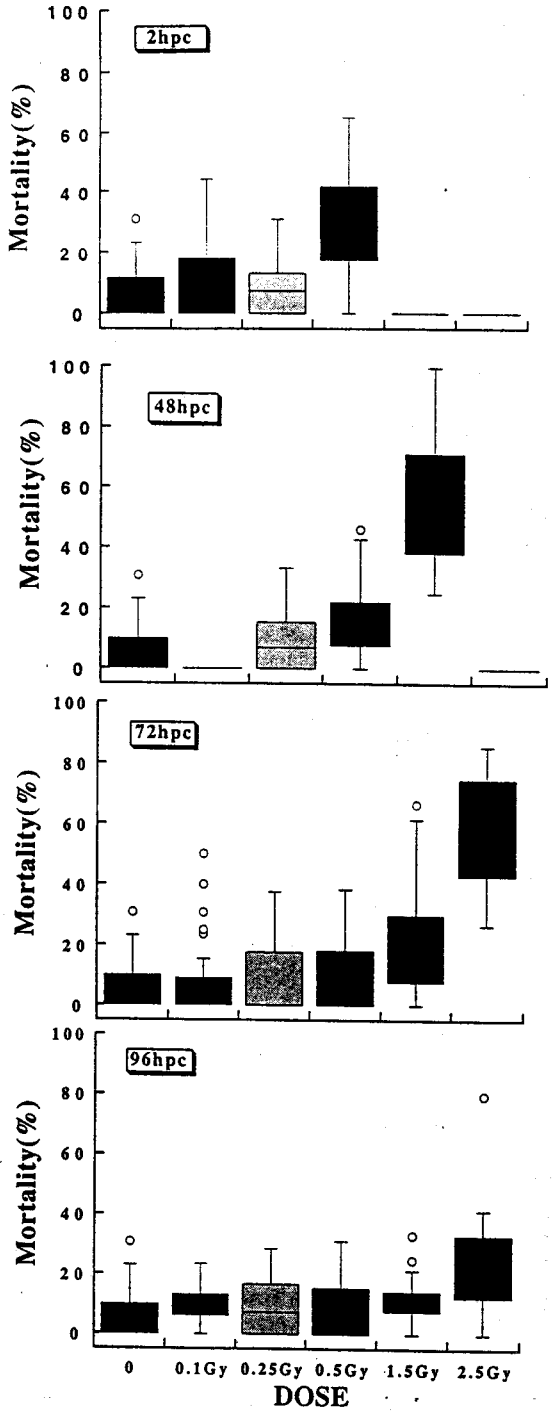

Fig. 2. Embryonic death of ICR mice irradiated at 2, 48, 72 and 96hpc in the preimplantation period. The dose-dependence was detected with statistical significance ($p<0.001$) among all dose groups by Kruskal Wallis test.

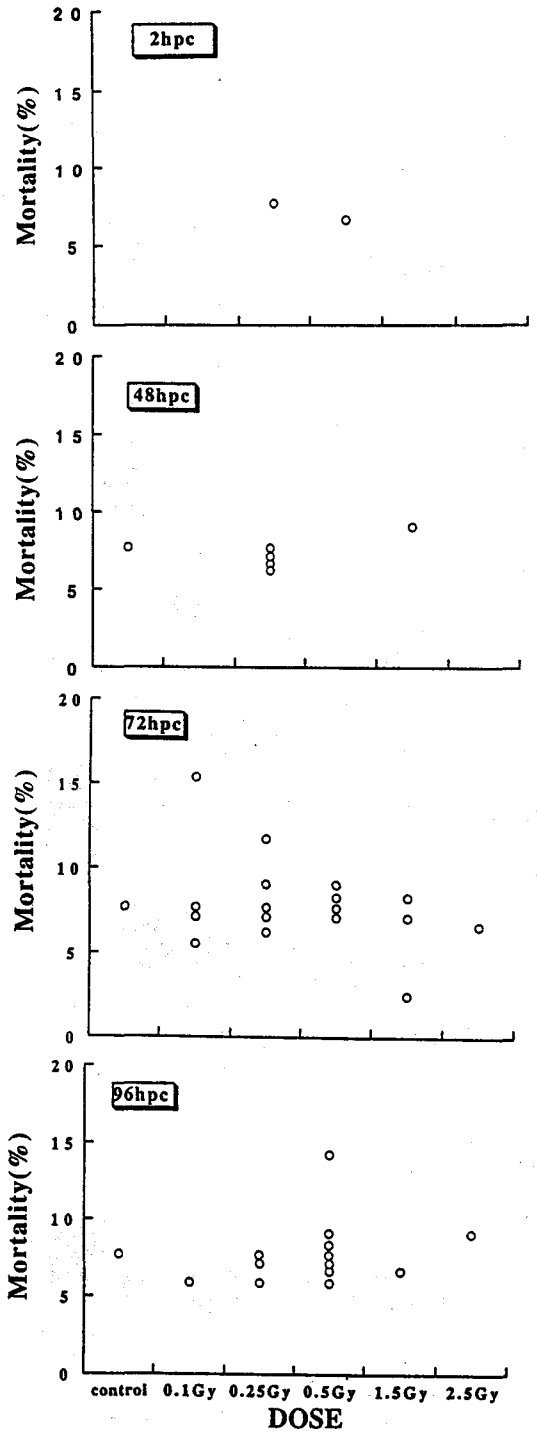

Fig. 3. Mortality of fetal stage of ICR mice irradiated at 2hpc in the preimplantation period. There were no statistical differences between control and each dose group.

Table 5. Numbers of fetuses bearing external malformations in mice irradiated at 2hpc during the preimplantation period.

Types of malformation	Control	0.1 Gy	0.25 Gy	0.5 Gy
Exencephaly	-	-	1	-
Hydrocephaly	-	-	-	-
Cleft plate	-	1	2	2
Ventral hernia	-	-	-	-
Chest hernia	-	-	-	1
Open eye	1	-	2	2
Anophthalmia	-	-	-	-
Abdominal hernia	-	-	-	1
Anomalies of tail	-	1	2	5
Polydactyly	-	-	-	1
polymelia	7	15	3	8
Total number of malformations	8	17	10	20
Percentage of malformataion(%)	1.42(%)	4.88(%)	3.54(%)	11.12(%)
Total number of live fetuses	561	348	282	177

Table 6. Numbers of fetuses bearing external malformations in mice irradiated at 2hpc during the preimplantation period.

Types of malformation	Control	0.25 Gy	0.5 Gy	1.5 Gy
Exencephaly	-	1	-	-
Hydrocephaly	-	-	-	-
Cleft plate	-	-	-	-
Ventral hernia	-	-	-	-
Chest hernia	1	-	-	-
Open eye	-	-	-	-
Anophthalmia	-	-	-	-
Abdominal hernia	-	-	-	-
Anomalies of tail	-	-	-	-
Polydactyly	-	-	-	-
polymelia	7	12	4	0
Total number of malformations	8	13	4	0
Percentage of malformataion(%)	1.42(%)	4.88(%)	1.90(%)	0(%)
Total number of live fetuses	561	266	210	110

Table 7. Numbers of fetuses bearing external malformations in mice irradiated at 72hpc during the preimplantation period.

Types of malformation	Control	0.1Gy	0.25Gy	0.5Gy	1.5 Gy	2.5 Gy
Exencephaly	-	1	3	-	-	1
Hydrocephaly	-	-	-	-	-	-
Cleft plate	-	1	3	1	1	1
Ventral hernia	-	-	2	-	-	-
Open eye	1	1	1	-	2	2
Anophthalmia	-	-	5	1	-	-
Abdominal hernia	-	-	-	-	-	1
Anomalies of tail	-	-	1	-	-	1
polymelia	7	18	15	12	7	8
Total number of malformations	8	20	30	14	10	14
Percentage of malformataion(%)	1.42 (%)	4.28 (%)	7.28 (%)	2.99 (%)	2.78 (%)	9.27 (%)
Total number of live fetuses	561	467	412	468	359	151

Table 8. Numbers of fetuses bearing external malformations in mice irradiated at 96hpc during the preimplantation period.

Types of malformation	Control	0.1Gy	0.25Gy	0.5Gy	1.5 Gy	2.5 Gy
Exencephaly	-	-	-	-	1	1
Hydrocephaly	-	-	-	-	-	-
Cleft plate	-	-	1	-	-	-
Ventral hernia	-	-	-	-	-	-
Open eye	1	-	1	-	1	-
Anophthalmia	-	-	-	-	1	2
Abdominal hernia	-	-	-	-	-	1
Anomalies of tail	-	2	1	-	-	4
polymelia	7	16	15	4	20	18
Total number of malformations	8	18	18	4	23	26
Percentage of malformataion(%)	1.42 (%)	6.84 (%)	7.53 (%)	1.84 (%)	9.31 (%)	12.80 (%)
Total number of live fetuses	561	263	239	217	247	203

Table 9. Threshold doses of embryonic death in mice irradiated at each stages during the preimplantation period.

Exposure time (hpc)	Threshould dose	
	LD_5	$LD_{10}\times 2/3$
2	0.2Gy	0.22Gy
48	0.33Gy	0.36Gy
72	0.53Gy	0.59Gy
96	1.18Gy	1.33Gy

Fetal Body Weight

The fetal body weight on day 18 of gestation are shown in the seventh column in Tables 1 to 4. The body weights of female and male control fetuses were 1.323 g and 1.376 g, respectively. There were no statistically significant difference in fetal body weights between irradiated and control mice. Also, There were no difference in sex ratios of mice between irradiated and control.

DISCUSSION

The regression curve of the mortalities of preimplantation and embryonic stages in mice irradiated at various periods of preimplantation fitted to Logistics models by SAS-LOGISTIC procedure are shown in Figs. 4 and 5. The threshold dose of embryonic death, which were assumed to be the same dose as 5% lethal dose of embryos, are shown in Table 9. In this study, the most sensitive period during preimplantation stage for embryonic death was 2 hpc, at which the embryos were one-cell stage and located at ampullary region of oviduct. One-cell stage embryo at 2 hpc have two haploid pronucleus in the cell. As shown in Figs 3 and 4, the sensitivity on embryonic death of embryos in early stage of preimplantation were higher than the those in later stage of preimplantation[23]. The sensitivities for embryonic

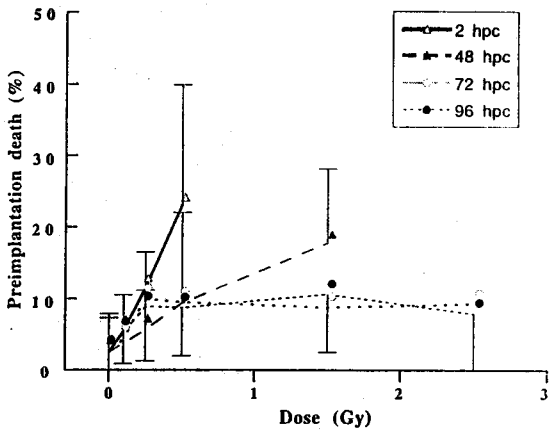

Fig. 4. Regression curves of preimplantation death rate in ICR mice irradiated at 2, 48, 72 and 96hpc during the preimplantation period. The dose-dependence was detected with statistical significance ($p<0.001$) among all dose groups by Kruskal Wallis test.

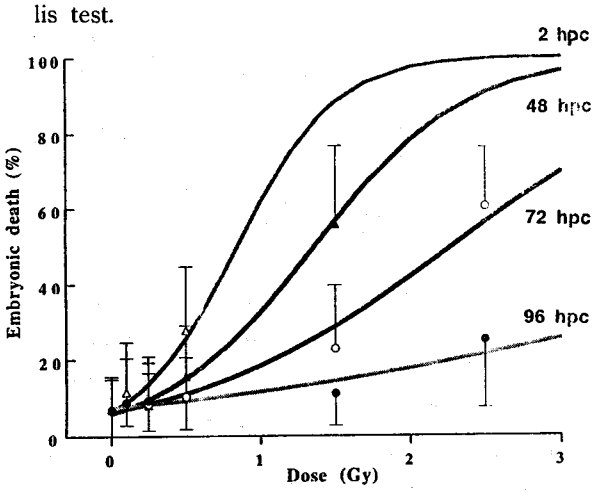

Fig. 5. Regression curves of embryonic death rate in ICR mice irradiated at 2, 48, 72 and 96hpc during the preimplantation period. The dose-dependence was detected with statistical significance ($p<0.001$) among all dose groups by Kruskal Wallis test.

death were decreased with developmental stage during preimplantation. In ICR mice, embryos on 48 hpc were 4- to 7-cell stage precompacted embryo, embryos at 72 hpc were about 30-cell stage embryos and embryos at 96 hpc were the blastocyst stage and shortly after the blastocyst has escaped from the zona. The threshold dose of embryonic death in mice irradiated at organogenesis were 1.4 Gy in our previous study[24]. Regarding the mortalities, the embryos irradiated at preimplantation stage were more sensitive than those irradiated during organogenesis.

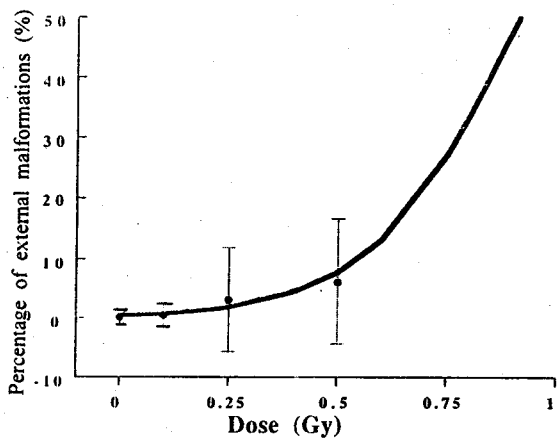

Fig. 6. Regression curve of frequencies of external malformation ICR mice irradiated at 2hpc in the preimplantation period. The dose-dependence was detected with statistical significance ($p<0.001$) among all dose groups by Kruskal Wallis test.

In this study, the many types of the external gross malformations such as exencephaly, cleft palated and anophthalmia were observed in the mice irradiated at 2, 72 and 96 hpc. Also, the threshold dose of external malformation in ICR mice irradiated at 2, 72 and 96 hpc were about 0.1 Gy[10,24]. This threshold of external malformations in ICR mice irradiated in organogenesis was lower than those in mice irradiated during organogenesis, which were between 0.5 to 1.0 Gy. Therefore, the sensitivity for external malformations in preimplantation stage embryos of the ICR mice was higher than those in organogenesis stage embryo. The studies carried out by Muller et al., were reported that

Heiligenberger mice had significant high sensitivity of gastroschisis for irradiation during preimplantation stage[9]. The types of external malformations were different between Heiligenberger mice and the ICR mice, however, the teratogenetic susceptibilities to radiation had both strain mice. From both studies, we did not believe that the embryo during preimplantation period were not susceptible to physical teratogens such as radiation. It was not sure whether the embryos during preimplantation period were susceptible to chemical and biological teratogenic agents, because the embryos in preimplantation stage were located in oviduct and surrounded by zona pellucida. The further investigation were needed to broke a dogma on the embryonic effects at preimplantation that there are no susceptibility for malformations.

In this study, no malformations were observed in the mice irradiated at 48 hpc, at which stage the embryos were about 6-cell stage precompacted embryos[25]. In precompacted embryos, all the cells formed the embryos would have totipotency and consequently damaged cells could be replaced by other cells with totipotency[26]. After 8-cell stage, compaction occurs among the cells and then each cell begins to have a specific function and morphology, therefore, many types of external malformation could occur. In the one-cell stage embryos, the causes of external malformation could be genetic changes of fertilized egg by teratogens such as radiation[27].

During preimplantation, a pregnant woman would not be aware of her pregnancy, thus there is a high possibility of exposure of developing embryos to radiation. On these bases, we strongly propose to follow the 10-day-rule, which means that a radiological examination involving the lower abdomen in a reproductive woman should be carried out only during the first 10 days after the onset of menstruation, and also which have been claimed to be more restrictive by many researchers[5].

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