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■ 論 文 ■

Combined Effects of Ionizing Radiation and Ultrasound on Malformation in ICR Mice at Organogenesis stage

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Abstract - Pregnant ICR mice were treated with 137Cs gamma-ray / ultrasound on day 8 of gestation. In combined treatments, pregnant mice were treated with both 1.5 Gy of radiation and 1.0 W/cm^2 ultrasound at time intervals of -1, 0 , 1, 3 and 6 hours. The mortalities and external malformations were investigated on day 18 of gestation. The threshold dose of mortality induced by radiation on day 8 of gestation was between 0.5 and 1.0 Gy, and that which was induced by ultrasound was between 1.0 and 1.5 W/㎠. The mortalities in the late-stage of gestation induced by combined treatment with radiation and ultrasound increased synergistically. The threshold dose of exencephaly and anophthalmia induced by radiation were between 0.5 and 1.0 Gy and between 1.0 and 1.5 Gy, respectively. Those of exencephaly and anophthalmia induced by ultrasound were between 1.0 and 1.5 W/㎠ and more than 1.5 W/㎠, respectively. In combined treatments, the incidence of exencephaly and anophthalmia were found to increase synergistically. In the mice treated with both agents at a time interval of one hour, the incidence of exencephaly and anophthalmia reached maximum levels.
Key word : Ultrasound, Radiation, Combined effects

요약 - 태생 8일째인 임신한 생쥐에 137-Cs 감마선과 초음파를 조사하였다. 복합조사의 경우 임신한 생쥐는 1.5 Gy 방사선과 1.0 W/cm^2 초음파로 -1, 0 , 1, 3, 6 시간 간격으로 조사하였다. 사망과 외부 기형은 태생 18일에 검진하였다. 방사선에 의한 태생 8일의 사망 임계값은 0.5에서 1.0 Gy 사이였으며, 초음파는 1.0에서 1.5 W/㎠ 사이였다. 태생 후반기의 사망은 방사선과 초음파의 복합 치료에 의해 상승적으로 증가되었다. 방사선에 의한 뇌탈출기형 및 무안구증의 임계값은 각각 0.5에서 1.0Gy사이와 1.0에서 1.5Gy 사이였다. 초음파에 의한 뇌탈출기형과 무안구증의 임계값은 각각 1.0에서 1.5 W/㎠ 사이와 1.5 W/㎠ 이상이었다. 복합치료의 경우는 뇌탈출기형과 무안구증의 빈도가 상승적으로 증가하였다. 한 시간 간격으로 두 가지 치료를 받은 생쥐에서 뇌탈출기형과 무안구증의 빈도가 최고치에 도달하였다.
중심어 : 초음파, 방사선, 복합 효과

INTRODUCTION

With the increasing utilization of radiation and nuclear energy, the probability of exposure to radiation has increased. Additionally, there are multiple factors in the environment, such as physical, chemical, and biological agents which may couple with radiation. For the most part, combinations of radiation with these agents enhances the biological effects of exposure to radiation. The probability of human beings being exposed simultaneously to two or more agents has increased greatly.

Embryos/fetuses are susceptible to ionizing radiation and other environmental agents, particularly embryos in the stages of pre-implantation and organogenesis[1-3]. At this stage, a pregnant woman would not be aware of her pregnancy; thus there is a high possibility for exposure of a developing embryo to radiation and other deleterious agents. Therefore, it is very important to examine how embryos are affected by two or more environmental agents during these stages.

There have been many experimental studies on the effects of each radiation and of the

other agents[2-4]. However, quantitative information concerning the combined effects of ionizing radiation and other agents on embryos/fetuses has been limited. Intrauterine mortalities and incidence of gross malformations were reported to increase synergistically in mice given a combined treatment of radiation and caffeine[5,6].

In this study, the combined effects of radiation and ultrasound on mice embryos on day 8 of gestation - the time of early organogenesis in mice - was investigated. In ICR mice, the neural folds and neural plates are differentiating and forming the neural tube in this stage. Radiation and ultrasound were selected as pathogenic agents on the embryos /fetuses in this study because both agents are physical agents, which affect the embryos directly. The initial cellular and tissue damage leading to developmental defects could be checked upon treatment.

MATERIALS AND METHODS

A closed colony of ICR (Crj:CD-1) mice purchased from Charles River Japan Company were used in this study. The mice were kept at a temperature of 21-23℃ and a relative humidity of 50 to 70% with a 12-hour light-dark cycle (starting at 6:00 a.m. and 6:00 p.m.), and were given free access to food (CA-1, CLEA Japan Inc.) and tap water.

One or two female mice of 8 to 12 weeks old were placed in the same cage with one male mouse of the same age range for a period of three hours between 6:00 a.m. to 9:00 a.m. The female mice were examined for vaginal plugs at 9:00 a.m. Those in which vaginal plugs were found were assumed to have been impregnated at 8:00 a.m., and that day was designated as day 0 of gestation[7,8].

Dams were treated with radiation/ultrasound on day 8 of gestation. Subjects were exposed to a single whole-body 137Cs-gamma ray at the dosages of 0.5, 1.0, and 1.5 Gy, with a dose rate of 0.2 Gy/min. Subjects were exposed to ultrasound for 10 min. at the power intensities of 1.0 W/㎠ and 1.5 W/㎠ , with a wave frequency of 1.0 MHz. Pregnant mice were put into plastic cages, which were specially designed for 4 x 2.5 ㎠ exposed area on abdomen and were exposed while immersed in water. The temperature of the bath water was maintained at 37±0.2℃. In combined treatments, Dams were treated with both 1.5 Gy of radiation and 1.0 W/㎠ of ultrasound at time intervals of -1, 0, 1, 3 and 6 hours. The indication of a time interval of "-1 hour" means that mice were exposed to ultrasound 1 hour prior to being irradiated, "0 hour" indicates that irradiation and exposure to ultrasound were conducted nearly simultaneously. For the remaining indications, irradiation was carried out first, followed by ultrasound after 1, 3, or 6 hours.

Pregnant mice were sacrificed on day 18 of gestation by the dislocation of the cervical spine and dissected at the abdomen to remove the fetuses. Dead fetuses were divided into two stage categories : implantation sites and placental remnants as early-stage death, and resorption of fetuses and maceration of fetuses as late-stage death. Live fetuses were removed from the uteri and the external malformations, fetal body weights and sex ratios were examined.

The rectal temperature of the pregnant mice exposed to the ultrasound was measured with a D620 thermometer(Tecno Seven Co.). The temperature of the ultrasonic bath water was always maintained at 37±0.2℃. And the rectal temperatures of pregnant mice immersed in bath water maintained at 41℃, without ultrasound exposure, were also measured.

The rectal temperatures were recorded every 10 seconds during the 10-minute treatment and then continuously measured for 15 minutes after the 10-minute treatment.

The reproductive parameters in each group, that is mortalities of embryo/fetus and so on, were analyzed by variation analysis with 95% or 99% confidence limits. The incidence of each external malformation in each group were analyzed by the Wilcoxon and the Kruskal Wallis tests with 95% or 99% confidence limits.

RESULTS

The total numbers of experimental pregnant mice, live fetuses, and fetuses bearing external malformations in this study are shown along with sex ratio and average of male and female fetal body weight in Table 1.

The early-stage mortality increased at dose of

Table 1. Fetal Death, Fetal Body Weight, Sex Ration and External Malformations Induced by Radiation/Ultrasound treated on Day 8 of Gestation.

Radiation (GY)	Ultrasound (W/cm)	Time Interval (hr)	No.of Dams	No. of Live feluse	Sex Ratio	Fetal Body Weight(g) Male	Fetal Body Weight(g) Female	No.of feluse Exencephay	No.of feluse malformation anophtalmia
0	0	-	24	275	0.515	1.354	1.309	0	0
1.5	0	-	20	260	0.471	1.345	1.237	0	0
1	0	-	25	297	0.475	1.320	1.267	9	0
1.5	0	-	20	237	0.498	1.240	1.177	14	26
0	1	-	22	238	0.475	1.317	1.296	0	0
0	1.5	-	20	223	0.515	1.451	1.370	9	0
1.5	1	-1	20	227	0.533	1.239	1.196	4	34
1.5	1	0	20	245	0.521	1.292	1.213	14	14
1.5	1	1	20	235	0.452	1.200	1.164	21	49
1.5	1	3	21	225	0.528	1.241	1.175	18	16
1.5	1	6	20	223	0.536	1.261	1.202	11	24
0	0	-	21	275	0.508	1.416	1.341	0	0

1.0 Gy of radiation ($p=0.151$), while no increase was found in the late-stage mortalities in all irradiated mice of radiation ($p=0.806$). Therefore, the threshold dose of early-stage mortality induced by radiation may be between 0.5 Gy and 1.0 Gy. There was a increase in the early-stage mortality at the power intensity of 1.5W/㎠ in comparison to that of control mice ($p=0.073$), while no increase was found in the late-stage mortalities in all mice treated with ultrasound. Therefore, the threshold dose of the early-stage mortality induced by ultrasound of ICR mouse embryos treated on day 8 of gestation may be between 1.0W/㎠ and 1.5W/㎠ of power intensity. The early and late-stage mortalities induced by the combined treatment of radiation at the dose of 1.5Gy and the ultrasound at power intensity of 1.0W/㎠ at time intervals of -1 hour, 0 hour, 1 hour, 3 hours and 6 hours are shown as box plot graphs in Fig. 1 and Fig. 2, respectively. In the early-stage mortalities, no statistically significant difference ($p=0.484$) could be found among all groups in comparison to that of mice exposure to 1.5Gy of radiation only, which was 8.5%. However, the late-stage mortality increased synergistically in the mice treated with both agents at time intervals of 1, 3, and 6 hours ($p=0.094$), in comparison to that of the 1.5Gy radiation treated mice, which was 1.9%.

The main types of external malformations in the ICR mice treated with radiation/ultrasound on day 8 of gestation were exencephaly and anophthalmia. The incidence of exencephaly in mice irradiated with radiation at dosages of 1.0Gy and 1.5Gy showed a statistically significant difference from that of the control mice ($p<0.001$). Also, the incidence of anophthalmia in mice irradiated with radiation at dose of 1.5Gy were statistically significantly different from that of control mice ($p<0.001$). The threshold dose of exencephaly by radiation was between 0.5Gy and 1.0Gy, and that of anophthalmia was between 1.0Gy and 1.5Gy. Embryos of the mice on day 8 of gestation were more prone to exencephaly than to anophthalmia upon radiation exposure. The incidence of exencephaly was increased statistically significantly ($p=0.029$) at the power intensity of 1.5W/㎠, while anophthalmia was not induced, even if exposed at power intensity of 1.5W/㎠. Therefore the threshold power intensity of exencephaly induced by ultrasound was between 1.0W/㎠ and 1.5W/㎠, and that for anophthalmia was more than 1.5W/㎠. The incidence of exencephaly and anophthalmia in mice induced by the combined treatment of radiation at a dose of 1.5Gy and the ultrasound at a power intensity of 1.0W/㎠ are shown in Fig. 3 and Fig. 4, respectively. In mice, treated

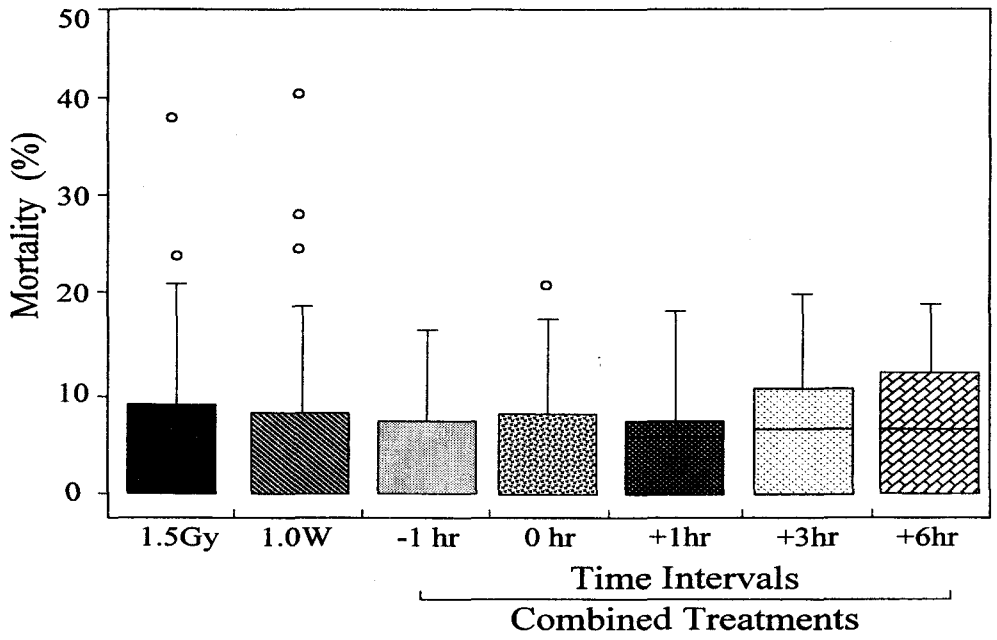

Fig. 1 Early stage mortalities of gestation in mice treated with radiation (1.5 Gy) and ultrasound (1.0 W/㎠) at time intervals of -1, 0, 1, 3, and 6 hours. There is no statistically significant difference among all groups in comparison to that of mice exposure of 1.5 Gy of radiation only.

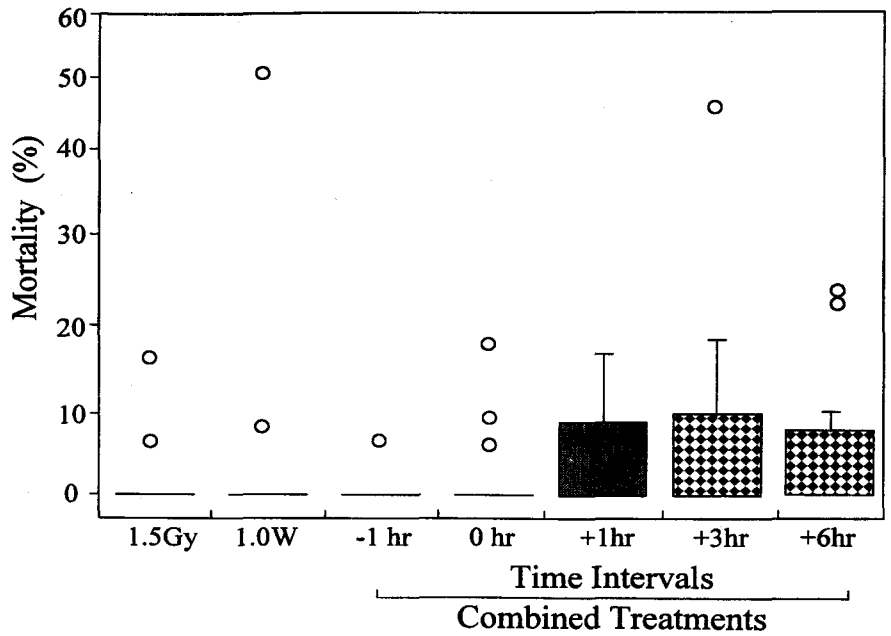

Fig. 2 Late stage mortalities of gestation in mice treated with radiation (1.5 Gy) and ultrasound (1.5 W/㎠) at time intervals of -1, 0, 1, 3, and 6 hours. Mortality increased synergistically in the mice treated with both agents at each time intervals in comparison to that of the 1.5 Gy treated mice.

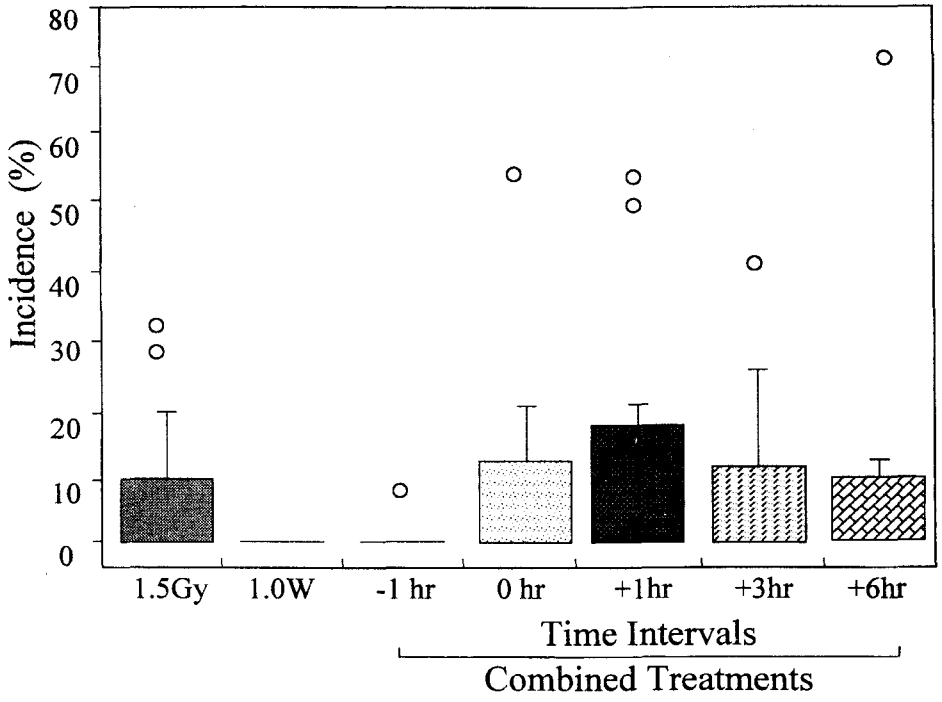

Fig. 3 Incidence of exencephaly in mice treated with radiation (1.5 Gy) and ultrasound (1.0 W/㎠) at time intervals of -1, 0, 1, 3, and 6 hours.

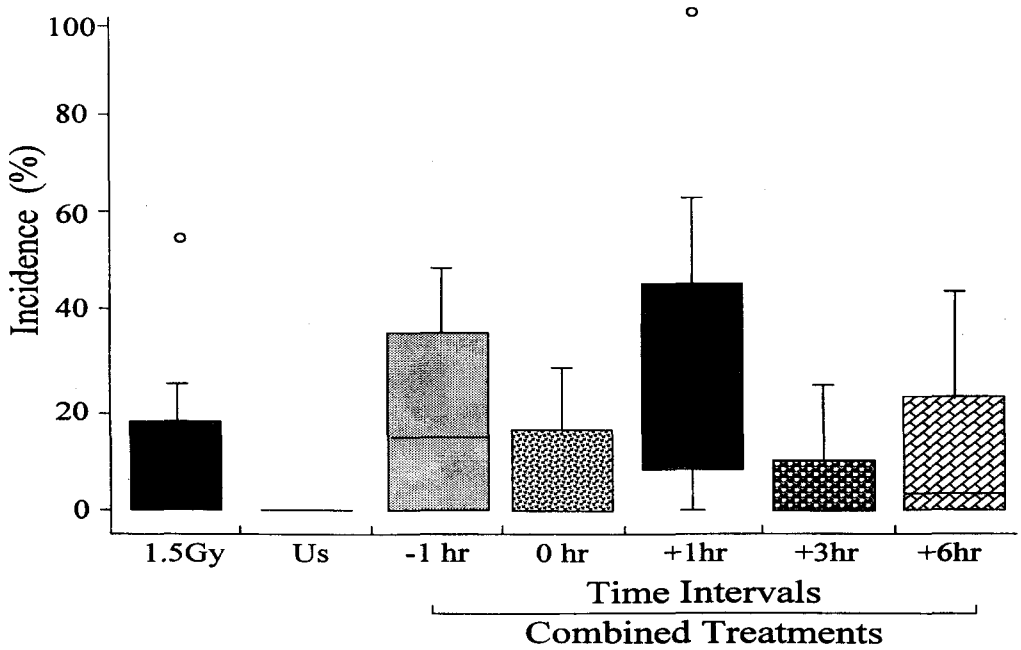

Fig. 4 Incidence of anophthalmia in mice treated with radiation (1.5 Gy) and ultrasound (1.0 W/㎠) at time intervals of -1, 0, 1, 3, and 6 hours.

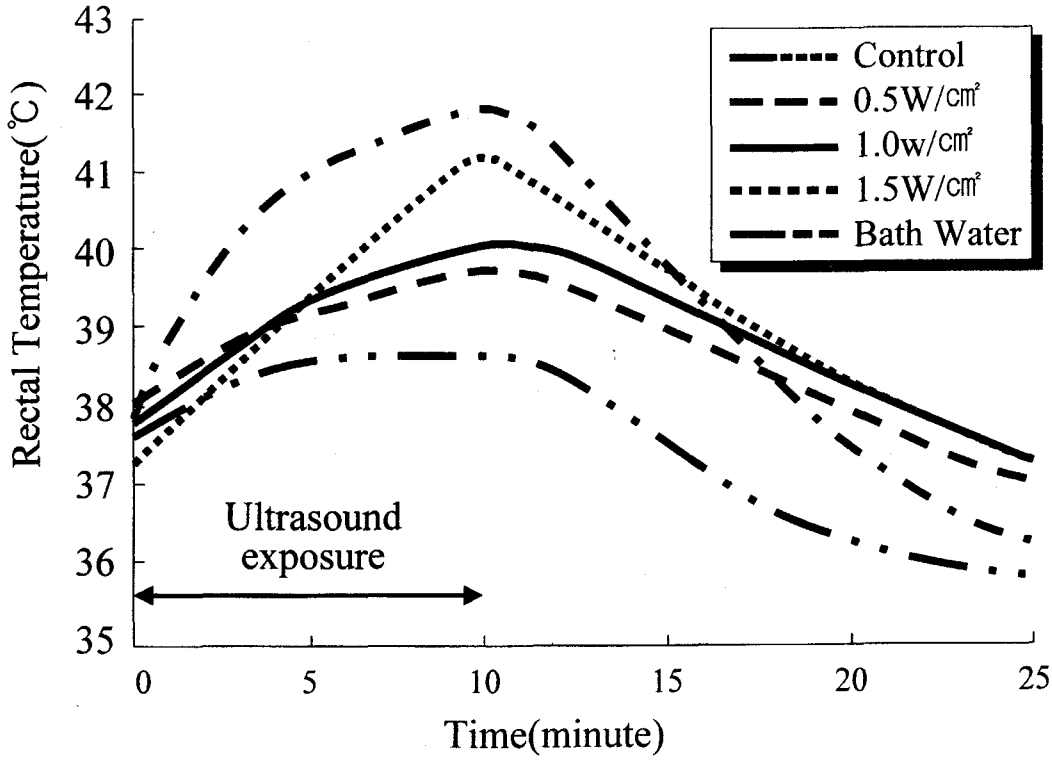

Fig. 5 Change of temperature of rectum in mice treated with ultrasound at power intensities of 0.5, 1.0, and 1.5 W/㎠, and in mice immersed in water bath at temperature of 41℃.

with both agents at a time interval of1hour, the incidence of each malformation-reached maximum levels. The incidence of anophthalmia in mice, induced by the combined treatment of both agents at a time interval of 1 hour, were statistically significantly increased (p=0.058) in comparison to mice treated with 1.5Gy of radiation only. It was evident that the incidence of exencephaly and anophthalmia of the fetuses were synergistically increased by the combined treatment of radiation at a dose of 1.5Gy and the ultrasound at a power intensity of 1 W /㎠ at a time interval of 1hour.

Fetal body weights were slightly decreased with the increase in dosage of radiation alone and were slightly increased with the increase of power intensity of ultrasound alone. In combined treatment, there were slight decreases that could be found in the mice treated at a time interval of 1 hour, but it was not statistically significant. Forfetalsexratios(Table 1), no differences were found between the control and the treated mice in this study.

Temperature variations of rectums in mice treated with ultrasound at the power intensities of 0.5, 1.0, and 1.5W/㎠ and in mice immersed in bath water at the temperature of 41℃ are shown in Fig. 5. In the group treated with power intensity of 1.5W/㎠, at which the external malformations were recognized (Table 1), the rectal temperature reached up to 41.6℃. In the sham control group, the temperature of the rectum reached just over 38℃.

DISCUSSION

In the normal development of the mouse, head process, neural plate, and optic sulcus begin to differentiate on day 7, 7.5 and 8 of gestation, respectively[9-12]. Several studies on the regional sequence of neural tube closure in mice revealed that fusions occur and progress at several places simultaneously. The initial appearance of the neural fold in ICR mice is

on day 7 and hour 17 of gestation. The first fusion begins on day 8 and hour 5 of gestation and is completed on day 9 and hour 21 of gestation[11]. In this study, exencephaly and anophthalmia were induced by the treatment with radiation/ultrasound on day 8 of gestation. At this time, the first fusion of the neural folds was observed to have started[11]; therefore treatment with radiation/ultrasound at this stage would interfere with the fusion and forming of the neural tube[13]. It was thus evident in this study that the mechanism of the induction of exencephaly via the treatment of radiation/ ultrasound on day 8 of gestation was due to the non-fusion of the neural tube.

In the mice treated with both agents at a time interval of 1hour, the frequencies of exencephaly and anophthalmia, which were 8.8% and 24.9%, increased significantly in comparison to the frequencies in mice treated with radiation alone,which was 5.8%. The late stage mortalities in the mice treated with a combined treatment of both radiation and ultrasound at time intervals of 1, 3, and 6 hours were 3.5%, 5.3%, and 3.2%, a significant increase was found in comparison to those in mice treated with radiation alone, which was 1.9%. It was considered that the fetuses bearing exencephaly had difficulty in surviving in the uteri due to exencephaly being a very severe malformation for fetuses. It appeared here that the fetuses bearing exencephaly would die in the late stage of gestation, and thus bring about an increase in the late stage mortalities. Therefore, in comparison to the group treated with radiation at dose of 1.5Gy, the frequencies of exencephaly in all groups treated with both radiation and ultrasound would increase significantly if fetuses bearing exencephaly survived until birth. From these results, it was concluded that the combined effects of external malformation, induced by the combined treatment of both radiation and ultrasound to embryos on day 8 of gestation, were synergistic and that the synergistic effects of combined treatment continued to effect untill the time interval of 6 hours.

The mechanism of ultrasound for external malformation may include pressure, tension, shearing stress, expansion, compression, velocity, and acceleration of cell death. In an absorbing medium, mechanical energy associated with ultrasound would be converted into heat and the temperature would thus rise, so these effects are considered to be thermal effects(14-17). In the mice treated with 1.5W/㎠, at which the external malformations were observed, the rectal temperature reached 41.6℃. We have observed, in the additional experiments, that when the temperature of the bath water increased to 41℃, the temperature of the rectum increased to 42℃, the same level reached by treatment with 1.5 W/㎠ of ultrasound (Fig. 5). However, no exencephaly could be found in pregnant mice of the total numbers of the 21 only immersed in 41℃ bath water(Table 1). From these results, the mechanism of ultrasound for external malformations was concluded to have no thermal effects of ultrasound but to have relevance to mechanical disturbance, e.g. pressure, tension, and so on. To make a final conclusion for the mechanism of ultrasound, further histological study is needed.

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