総　説

Inhibition of X-radiation Induced Malformation Damage in Mice by TMG (*Water-soluble Derivative of Vitamin E*)

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Key Words: TMG, Organogenesis stage, Radioprotective effects, Malformation, Embryonic death, Cellular level

Abstract

The ICR mice were used on organogenesis stage in fetuses, and it was made clear experimentally about the mechanism of the protecting effect of the vitamin E derivantive (TMG; 2-(α-D-Glucopyranosyl) Methyl-2, 5, 7, 8-Teramethylchorman-6-OL) to the radiation in this study. It paid attention to the radiation, and it was examined about the radioprotective effects of TMG to the induced malformation. The excuse of the protection of the highest fetus malformation of the sensibility was examined the research as a radioprotective effects. The malformation to the radiation of the organogenesis stage, skeletal malformation, and the examination of the cellularis level of the embryo was done in this research. And it was analyzed and observed experimentally about the mechanism of the protection effect of TMG against the fetus malformation in radiation. Therefore, the following research was done in this research to provide the foundamental data of the radiation protection agent of TMG. It was made clear that TMG had radioprotective effects against the embryonic death. Radioprotective effects were recognized as the teratogenesis rate in some significant before the exposure by TMG administration. As for the skeletal malformation and the fetal body weight as well, radioprotective effects were made clear. Radioprotective effects was made clear in the same way as the whole body level as for the cellular level as well to the radiation exposure by TMG administration.

INTRODUCTION

The aim of radiation safety and the protection are a human and the safety security of the environment. The profit which radiation brought to the human is very big. The radiation has even harmful factor for the human besides one case. Nuclear power at the industry territory has been contributed large for the use to the radiation peace. And, as for the present clinical medicine, the medical care technique, which as for the medical territory as well, radiation was used for, can't be missed. But, there are many problems that a radiation protection plan at the nuclear power station hasn't been solved internationally in such actual circumstances, too. Moreover, fetal effects by the radiation for the medical use are not only the medical people but also, and it is paid attention to socially. The mission of the nuclear power station is the subject that it is critical to make protection of radiation to the people engaged in the nuclear power station and the periphery inhabitant a perfect thing. It is the goal that it is the biggest whether to make radiation protection from the internal exposure of the human and the external exposure. After effects on man to the radiation are thought about, the individual of which sensibility is the highest is a fetus[1].

According to Rema, Alpha-tocopherol monoglucoside (TMG), a water-soluble derivative of α-tocopherol, has been examined for its ability to protect DNA against radiation-induced strand breaks. Gamma radiation, up to a dose of 6Gy (dose rate, 0.7Gy/minute), induced a dose-dependent increase in single strand breaks (SSBs) in plasmid pBR322 DNA. TMG inhibited the formation of γ-radiation induced DNA single strand breaks (SSBs) in a concentration-dependent manner; 500 μM of TMG protected the single strand breaks completely. It also protected thymine glycol formation induced by γ-radiation in a dose-dependent manner, based on an estimation of thymine glycol by HPLC[2].

By the reactions between tocopherol monoglucoside (TMG), a water-soluble vitamin-E derivative, with $Br_2^{\bullet -}$, $N_3^{\bullet}$, $(SCN)_2^{\bullet -}$, $NO_2^{\bullet}$, $OH^{\bullet}$ and various halogenated peroxyl radicals were examined using a pulse radiolysis technique. The results demonstrate that TMG forms a stable phenoxyl radical at pH$>$6.8. The thus-formed phenoxyl radical shows pH-dependent decay kinetics and is disproportionated by 2nd order kinetics at pH 2.3. It was observed that the TMG reactivity towards a halogenated peroxyl radical increases with the number of halogen atoms at the carbon atom having a peroxyl group. The reaction between the TMG phenoxyl radical and ascorbic acid was also examined using a pulse radiolysis technique. The results indicated that the TMG phenoxyl radical is repaired by ascorbate. Kinetic studies indicate that TMG may act as an antioxidant to repair free-radical damage to some biologically important compounds. The one-electron reduction potential for TMG was found to be 0.522 V$\pm$0.06 vs. NHE[3].

According to Sudhir, alpha-TMG is a novel water-soluble derivative of Vitamin E that has shown excellent antioxidant activity. The parent compound has demonstrated protection against radiation induced chromosomal damage in vivo. Hence, the preliminary experiments to determine the radioprotective activity of alpha-TMG were carried out in adult Swiss albino mice. Acute toxicity of the drug was studied taking 24 h, 72 h and 30 day mortality after a single intraperitoneal injection of 500–2000 mg/kg body weight of the drug. The drug LD (50) for 24 h, 72 h and 30 days survivals

were found to be 1120 and 1000 mg/kg body weight, respectively. The optimum time of drug administration and drug dose-dependent effect on in vivo radiation protection of bone marrow chromosomes was studied in mice. Injection of 600 mg/kg of the drug 15 min before or within 5, 15 or 30 min after 3Gy whole body gamma radiation resulted in a significant decrease in the aberrant metaphases percent at 24 h post-irradiation; the maximum effect was seen when the drug was given immediately after irradiation. Injection of 200-800 mg/kg TMG within 5 min of irradiation with 3Gy produced a significant dose-dependent reduction in the radiation induced percent aberrant metaphases and in the frequency of micronucleated erythrocytes at 24 h after exposure, with a corresponding decrease in the different types of aberrations. The optimum dose for protection without drug toxicity was 600 mg/kg body weights. At this dose, TMG produced 70 and >60% reduction in the radiation induced percent aberrant metaphases and micronucleated erythrocytes, respectively. The high water solubility and effectiveness when administered post-irradiation favor TMG as a likely candidate for protection in case of accidental exposures[4].

According to Yoshida, a novel vitamin E derivative that is freely soluble in water, 2-(alpha-D-glucopyranosyl) methyl-2, 5, 7, 8-tetramethylchroman-6-ol (TMG), was evaluated for ability to inhibit development of atherosclerosis in Watanabe heritable hyperlipidemic (WHHL) rabbits or cholesterol-loaded New Zealand White rabbits. Although TMG rapidly entered the circulation blood after oral administration, the blood TMG concentration remained low, while neither TMG nor its metabolites appeared in the low-density lipoprotein (LDL) fraction. TMG did not decrease serum total cholesterol and the various lipoprotein-associated cholesterol fractions (very LDL-, or high-density lipoprotein- (HDL) cholesterol). TMG reduced the serum concentration of thiobarbituric acid-reactive substances (TBARS; an index of lipid peroxidation) in cholesterol-loaded rabbits but not WHHL rabbits. Nonetheless, TMG inhibited aortic atherosclerosis as effectively as probucol in both models. Our results indicated that TMG opposes progression of atherosclerosis not only by preventing oxidation of LDL, but also by presently unknown mechanisms. Even an antioxidant with no uptake by LDL apparently can inhibit development of atherosclerosis despite a very low serum concentration[5].

According to Murase, a novel TMG, has excellent water-solubility ($>1\times10[3]$ mg/ml). The antioxidant activity of TMG was investigated. Kinetic studies of the inhibition of radical-chain reaction of methyl linoleate in solution demonstrated that the peroxyl radical-scavenging activity was not changed by the replacement of phytiyl side chain of vitamin E to glucosyl group. TMG acted as an effective inhibitor on lipid peroxidation of egg yolk phosphatidylcholine (PC)-liposomal suspension induced by a water-soluble and a lipid-soluble radical generator, 2,2'-azobis (2-amidinopropane) dihydrochloride (AAPH) and 2,2'-azobis (2,4-dimethylvaleronitrile) (AMVN). Its effectiveness was higher than that of ascorbic acid (AsA) when liposomal suspension was exposed to a lipid-soluble radical generator, AMVN. TMG also showed an excellent antioxidant activity on cupric ion-induced lipid peroxidation of PC-liposomal suspension, and suppressed the oxidation of rat brain homogenate, which contained trace level of iron ion. On the other hand, AsA acted as a prooxidant on both the cupric ion-induced liposomal peroxidation and the oxidation of rat brain homogenate. When human

plasma was exposed to either AAPH or AMVN, the accumulation of cholesteryl ester hydroperoxides was retarded by the addition of TMG[6].

Radiological safety and the goal of the protection are a human and the safety security of the environment. Much research is needed, and it is almost being made clear until now as for the relations between the fetal death and the radiation. There is even stage when expectant mother doesn't notice pregnancy mainly until the organogenesis in the stage when malformation is induced. Because it is the decisive stage which rational symptom is not like this in, the potential that an effect is taken from the radiation is high. The malformation due to the radiation in the environmental agent, external malformation, skeletal malformation are observed by using the ICR mice used for the malformation study abundantly in this determination stage. Moreover, observation in the fetus of cellular level to the radiation has been done and examined about the radiation protecting effect of TMG. It is being made clear with TMG to be similar to the mechanism of the vitamin E and to have the protection effect which faces free radical by the experiment of In vitro by the preceding research[8),9)]. An excuse as a radioprotective agent of the effect in the radiation to fetus is examined experimentally by using TMG in this research.

MATERIAL AND METHODS

1. EXPERIMENTAL ANIMALS AND MATING PROCEDURE

We used ICR mice housed at a temperature of 21-23°C and a relative humidity of 50 to 70% with a 12-hour light-dark cycle (lights on at 8 : 00 and off at 20 : 00).

We used ICR mice from the Japan National Institute of Health. Color is albino, and the generative and the development of are good. And it is being used for the bioassay and researches others widely in Japan.

A closed colony of ICR mice was purchased from SLC Japan, Inc.

The mice were given free access to food (CA-1, CLEA Japan Inc.) and to tap water. One or two female mice and one male mouse of the same age range were mated for only three hours from 8 : 00 to 11 : 00. The female mice in which vaginal plugs were detected were assumed to have become pregnant at 10 : 00[10].

2. IRRADIATION WITH X-RAYS

The pregnant mice were placed in plastic cages for exposure, and were treated with a single whole-body X-radiation at 2Gy with a dose rate of 35 cGy/min. We used a 225 kV X-radiation source (Philips). The time of exposure for embryos was 192 hpc.

3. TMG INJECTION METHODS

The pregnancy mice of (the organogenesis when it is formed nervous facies) was put in the exposure gage after the conception coming into existence eight days, and exposure procedure went by the dosage rate 0.35Gy/min through the 2.0Gy radiation. As for the injection method of TMG, venter administered TMG 600 mg/kg (isotonic sodium chloride solution 1.5 cc) to the pregnancy mice after the conception coming into existence 8 days. Because metabolism in the organism was very early in comparison with the vitamin E, TMG went for this disposal the radiation exposure five minutes ago[8),9)].

4. OBSERVATION OF EXTERNAL and SKELETAL MALFORMATIONS AND OTHER EFFECTS

After irradiation, the pregnant mice were sacrificed by cervical dislocation on day 18 of gestation and the total numbers of corpus luteums 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. Skeletal malformation went in accordance with the Jacobsen procedure[11]. The examination of the cellular level did radiation exposure to the pregnancy mice after the conception coming into existence eight days and embryos was taken out from the maternal mouse after six hours. It was made to fix one temporal in the fixative, and embryos taken out went in accordance with the HE staining method after the fixation's method.

5. STATISTICAL METHODS

In studying teratological effects, it is not appropriate to consider the fetus/embryo to be an experimental unit[12]. Instead, the litters (pregnant mice) were used as the experimental unit in the statistical analysis of the experimental data. In the per litter analysis, an average fetal response within a litter was calculated. For statistical tests, we used non parametric methods, Wilcoxon tests for comparisons between two groups[13]. This is because the embryo/fetus binary response data do not show a normal distribution.

And, as for the fetal body weight, a t t-test test was done to follow Gauss distribution. About statistical analysis to the effect of the cellular level, a statistical test to pyknosis cell and micronuclei used a Wilcoxon test[13].

RESULTS

Preimplantation death

It is shown to Table1 about the preimplantation death rate. Statistical significant difference was recognized between the preimplantation death rate of the 2.0Gy group and the control group ($P<0.01$). Preimplantation death rate faced 3.9% of the control group, and it was sham control group 16.9% 2.0 Gy group 11.9% TMG+2.0Gy group 15.7% 2.0Gy+

Table 1. Embryonic/fetal death and fetal body weight of ICR mice irradiated at 192 hpc during organogenesis period.

Treatment (Gy)	Number of Dam	Primplantation deaths		Embronic deaths		Fetal deaths		Live fetuses		Litter size		No. of Implantation (%)	
		Number	%	Number	%	Number	%	Number	%	Mean	SD	Male	Femele
Control	20	13	3.94	18	5.68	5	1.58	294	92.75	14.7	2.83	1.23±0.12	1.16±0.14
Sham control	20	56	16.92[a]	8	2.91	8	2.91	259	89.62	12.95	2.8	1.20±0.19	1.16±0.17
2Gy	20	29	11.89[a]	134	62.33[b]	11	5.12[a]	70	32.5[b]	3.5[b]	3.46	1.12±0.13[c]	1.04±0.13**
TMG+2Gy	20	36	15.72[a]	38	19.69[a,b]	8	4.15[a]	147	76.17[a,b]	7.35[a,d]	4.76	1.19±0.13	1.12±0.15
2Gy+TMG	20	19	8.09	187	89.57[b]	6	2.78	23	10.65[b]	1.15[b]	1.53	1.02±0.17[c]	0.97±0.09**

M : Male, F : Female

a Significant difference against control by Wilcoxon of nonparametric test, Significant at a 5% probability level.

b Significant difference against control by Wilcoxon of nonparametric test, Significant at a 1% probability level.

c Significant difference against control at a 1% probability level by use of students t-test.

d Significant difference against 2Gy by Wilcoxon of nonparametric test, Significant at a 5% probability level.

TMG group 8.1%, respectively, by other exposure groups. Because preimplantation death is different from the radiation exposure stage there isn't this causal association with the range of error of the experiment. Moreover, it is absorbed, and it is thought that it was judged with the death preimplantation death by the metabolism of which implantation sites is early.

Embryonic death

Implantation sites, placental remnant, absorption embryos were handled as an embryonic death. Embryonic death is the death which happened from the post conception 4.5 days by 13.5 days[1]. It is shown to Table1 and Fig. 1 about the embryonic death rate. The embryonic death rate of the control group faced 5.7% and 2.9% a sham control group a TMG+2.0Gy group has were 19.7% respectively. The statistical significant difference wasn't recognized between the embryonic death rate of the Sham control group and the TMG+2.0Gy group and the embryonic death rate of the control group. But, the embryonic death rate of the 2.0Gy group and the 2.0Gy+TMG group was 62.3%, 86.6% respectively. The statistical significant difference was recognized between the embryonic death rate of the 2.0Gy group and the 2.0Gy+TMG group and the embryonic death of the control group ($P<0.001$). Therefore, if TMG wasn't administered, the embryos to death beyond the haploid number, which did implantation, were found out in the exposure beyond 2.0Gy.

Fetal death

The thing, which was a macerate fetus in the observation day after the conception18 days, was handled as a fetal death. Fetal death is thought to be the death, which happened after the conception 14 days. The result of the fetal death rate is shown to Table1. The fetal death rate of the control group was sham control group 2.9% 2.0Gy group 5.1% TMG+2.0Gy group 4.2% 2.0Gy+TMG group 2.8% in 1.6%. The statistical significant difference wasn't recognized between the fetal death rate of the control group and the fetal death rate of each exposure group. Therefore, though TMG was used,

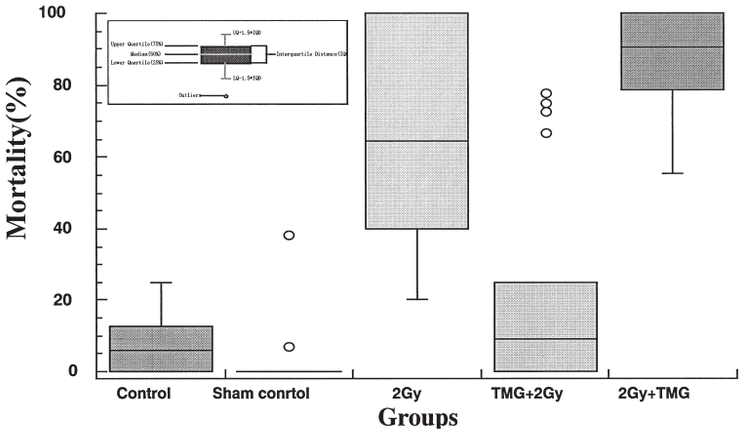

Fig. 1. Embryonic death of ICR mice irradiated at organogenesis period.
The 2Gy and 2Gy+TMG group were detected with statistical significance ($p<0.001$) in comparison with control and sham control groups by Wilcoxon test.

it was found out that some thought differences weren't recognized to the Control group in the exposure beyond 2.0Gy. Because the incidence of the embryonic death was very high, it isn't thought that it was a difference for the fetal death as a one of the reason.

External malformation

The number of the development of the malformation is shown to Table2 and Fig. 2. The modality of the malformation that it is induced in each exposure group is exencephaly, cleft palate, abdominal-hernia, open eye, gastroschisis, and anomalies of tail. The development frequency of the malformation is control group 1.0-% sham control group 0.4% 2.0Gy group 57.1% TMG+2.0Gy group 35.4% 2.0Gy+TMG group 56.5%. It faced as for the malformation, which occurred in the control group though it was the unusual chisels for medical use of the caudal, and the sham control group didn't induce malformation. Statistical significant difference wasn't recognized in the malformation incidences between of the control group and the sham control group. Statistical significant difference was recognized as the development frequency of the deformity of the 2.0Gy group (57.1%) in comparison with the development frequency of the Control group (1.0%) ($P<0.001$). Statistical significant difference was recognized as the teratogenesis rate (35.4%) of the TMG+2.0Gy group as well in comparison with it of the control group. Statistical significant difference was recognized as the teratogenesis rate (56.5%) of the 2.0Gy+TMG group to it of the control group. As for the malformation, statistical significant difference was recognized as the control group from the above fructification to the sham control group in all the exposure groups. But, the TMG+2Gy group in less than 2/3 recognized decre-

Table 2. Numbers of fetuses bearing external malformations in mice irradiated at 192hpc during the organogenesis period.

Types of malformation	Control	Sham Control	2Gy	TMG+2Gy	2Gy+TMG
Exencephaly	0	0	9[b]	30[b]	1
Hydrocephaly	0	0	3[a]	1	3[a]
Cleft palate	0	0	2	1	1
Open eye	0	0	0	4[a]	0
Anophthalmia	0	0	2	14[b,c]	2
Abdominal-hernia	0	0	1	0	0
Anomalies of tail	0	0	22[b]	2	6[b]
polymelia	0	1	0	0	0
Total number of malformations	0	1	39	25	13
Fryquency of malformations (%)	0	0.62	55.71[b]	17[b,c]	56.52[b]
Total number of dams	20	20	20	20	20
Total number of live fetuses	294	259	70	147	23

a Significant difference against control by Wilcoxon of nonparametric test, Significant at a 5% probability level.

b Significant difference against control by Wilcoxon of nonparametric test, Significant at a 1% probability level.

c Significant difference against 2Gy by Wilcoxon of nonparametric test, Significant at a 5% probability level.

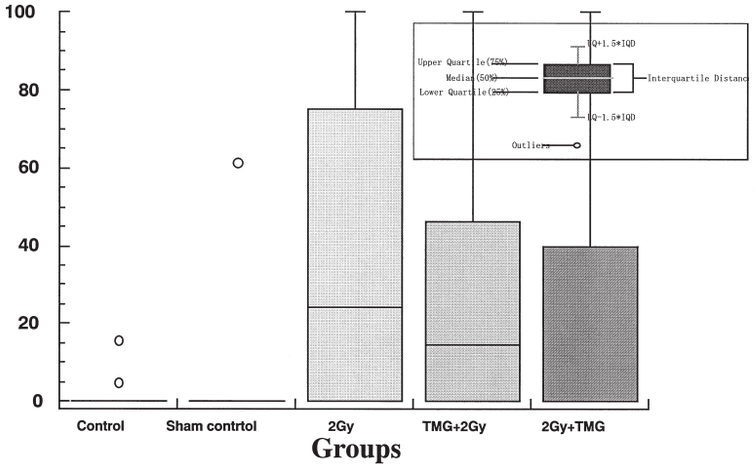

Fig. 2. Malformation incidence of ICR mice irradiated at organogenesis period.
Each irradiation group were detected with statistical significance ($p < 0.001$) among all dose groups by Wilcoxon test.

ment though it was the highest by the 2.0Gy group and the 2.0Gy+TMG group when it was seen from the teratogenesis rate. Because there are many examples, which died because it had malformation in the death, as for the effects of the malformation and the influence of each death, it is separated, and you must not think about it.

Skeletal malformation

The number of the development of the skeletal malformation is shown to Table3 and Fig. 3. The modality of the skeletal malformation that is induced in each exposure group is the following. The development frequency of the skeletal malformation is control group 0.1% sham control group 0% 2.0Gy group 47.1% TMG+2.0Gy group 13.6% 2.0Gy+TMG group 43.5%. It faced as for the skeletal malformation that occurred in the control group though they were fused rib, malpositioned thoracic vertebra, absent caudal centrum, and skeletal malformation didn't occur in the sham control group. It appeared acrania, craniofenestria, and bipartite ossification of sternebral, unossified sternebra, misshapen sternebra, fused rib, malpositioned thoacic vertebra, and malpositioned lumber vertebra, absent caudal centrum by the 2.0Gy group. Development frequency was high with 47.1%, and statistical significant difference was recognized as between the skeletal malformation incidence of the control group, too ($P < 0.01$). Though bone of acrania, absent maxilla, unossified clavicle, incomplete ossification of rib, full supernumerary rib appeared by the TMG+2.0Gy group and development frequency was compared with the development frequency of the 2.0Gy group in 13.6% and it was poor.

Statistical significant difference was recognized as this as well between the skeletal malformation incidences of the control group ($P < 0.01$). It appeared acrania, bipartite ossification of sternebral, misshapen sternebra, malpositioned thoacic vertebra, absent caudal centrum by the 2.0Gy+TMG group, and development frequency was comparatively high with 43.5%. Statistical significant

Table 3 Numbers of fetuses bearing skeletal anomalies in mice irradiated at 192hpc during the organogenesis period.

Types of malformation	Control	Sham Control	2Gy	TMG+2Gy	2Gy+TMG
Acrania	0	0	7[b]	15[b]	3[a]
Craniofenestria0	0	1	0	1	0
Absent maxilla	0	0	0	1	0
Unossifies clavicle	0	0	0	1	0
Biqartite ossification of sternebral	0	0	9[b]	0	1
Unossified sternebra	0	0	4[a]	0	0
Misshapen sternabra	0	0	2	0	1
Incomplete ossification of rib	0	0	0	2	0
Fused rib	2	0	2	0	0
Full supernumerary rib	0	0	0	1	0
Malpositioned thoacic vertebra	1	0	3[a]	0	1
Malpositioned lumber vertebra	0	0	2	0	0
Absent caudal centrum	1	0	3[a]	0	4[a]
Total number of skeletal anomalies	4	0	33	20	10
Frequency of skeletal anomalies (%)	0.13	0	30.98[b]	10.22[a,c]	19.75[b]
Total number of dams	20	20	20	20	20
Total number of live fetuses	294	259	70	147	23

a Significant difference against control by Wilcoxon of nonparametric test, Significant at a 5% probability level.

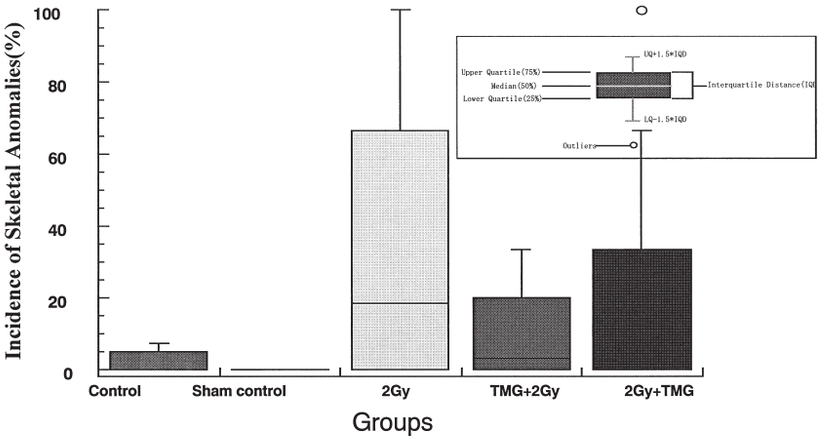

Fig. 3. Skeletal anomalies of ICR mice irradiated at organogenesis period. The radiation and TMG plus radiation group were detected with statistica significance ($p<0.001$) among all dose groups by Wilcoxon test.

difference was recognized as these as well between the skeleton malformation incidences of the control group ($P<0.05$). As for the skeletal malformation, statistical significant difference was recognized as the control group from the above fructification to the sham control group in all the exposure groups. But, it was found out that it had the incidence of the skeletal malformation restrained by administering TMG before the 2.0Gy exposure.

Fetal body weight

After one measured fetal body weight respectively and every mother's average was asked, the fetus' average weight of each exposure group asked a mean by the male and female of each exposure group. The fetal body weight of each exposure group is shown to Table1 and Fig. 4. Both significant difference of the fetal body weight decrement was recognized in it of the 2.0Gy group and the 2.0 Gy+TMG group in embryonic age 18-day fetal body weight toward the body weight of the Control group and the Sham control group of the male female ($P<0.001$). But, some thought differences weren't recognized in it of the TMG+2.0Gy group in the fetal body weight decrement to the body weight of the control group and the sham control group. And, when a male and female ratio was compared, there was little fetal body weight of the female more than the fetal body weight of the male in about the circa 0.05 g as for which as well as well.

A radiation effect in the cellularis level of the embryo

As for pyknosis, the fraction of pyknosis cell to the embryo when 2.0Gy-radiation exposure was done after the conception 8 days and micronuclei is shown to Fig. 5. A Wilcoxon test was used for the significant difference test of the control group and each exposure group. Statistical significant difference wasn't recognized between pyknosis of the control group and pyknosis of the sham control group as a result of the observation. But, statistical significant difference was recognized between pyknosis of the control group and pyknosis of the 2.0Gy group ($P<0.001$). Significant difference was recognized in the same way between pyknosis of the 2.0Gy+TMG group as well. ($P<0.001$). But, statistical significant difference wasn't recognized in pyknosis of the TMG+2.0Gy group toward pyknosis of the control group. Therefore, it was made clear that TMG had the effects that pyknosis is restrained. And, existence was recognized with it little in other exposure groups though it didn't exist about micronuclei in the control group.

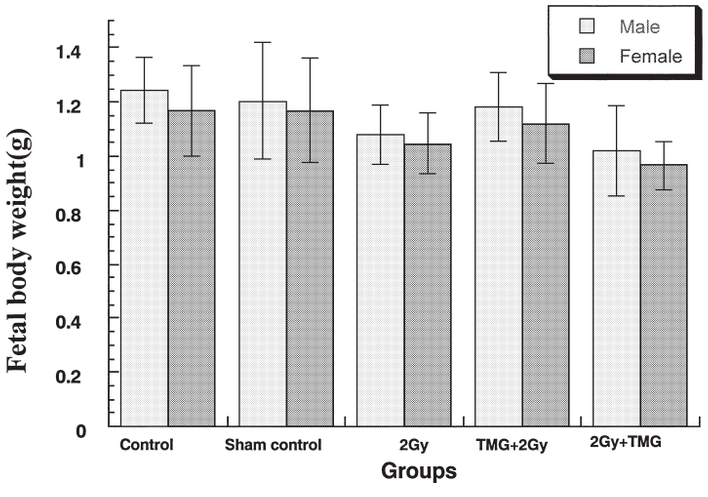

Fig. 4. Fetal body weight of ICR mice irradiated at organogenesis period.
2.0Gy and 2Gy+TMG groups were detected with statistical significance ($p<0.05$) in comparison with control and sham control groups by t-test.

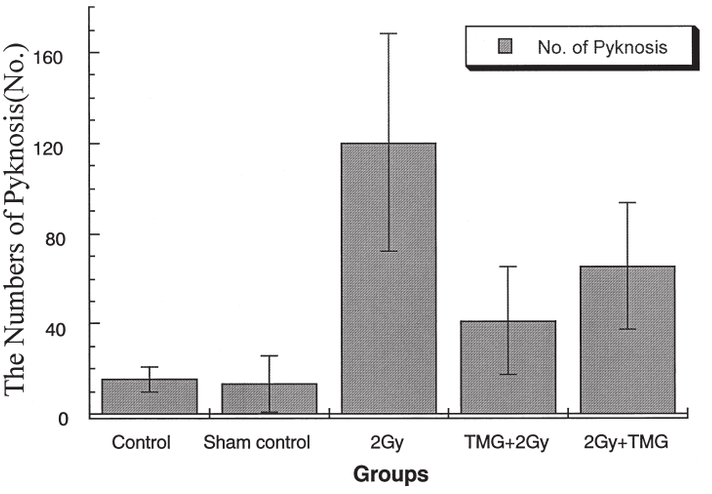

Fig. 5. The pyknosis of mice embryos mice irradiated at organogenesis period.
The radiation, TMG plus 2Gy and 2Gy plus TMG groups were detected with statistical significance ($p<0.001$) among all treatment groups by Wilcoxon test.

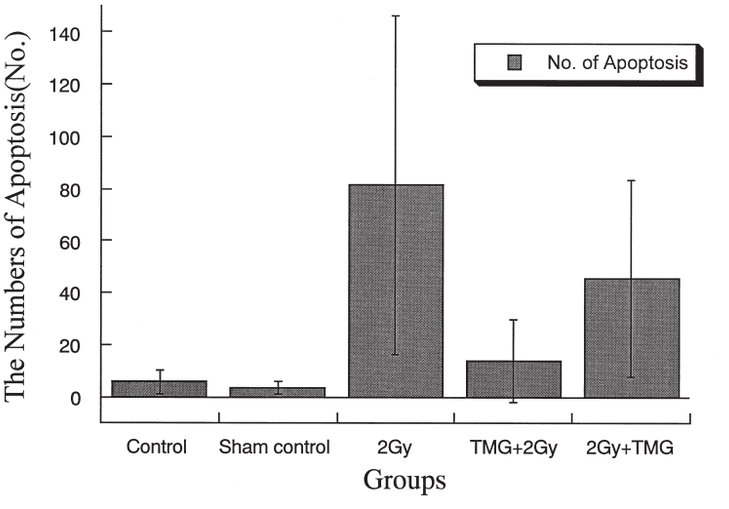

Fig. 6. The apoptotic cell of mice embryos mice irradiated at organogenesis period.
The radiation, TMG plus 2Gy and 2Gy plus TMG groups were detected with statistical significance ($p<0.001$) among all dose treatment groups by Wilcoxon test.

The effect of apoptosis

The number of apoptosis of each treatment group when radiation exposure was done after a conception 8 days is shown to Fig. 6. And, It was in comparison with the control group and the sham control group by the 2Gy group ($P<0.001$), the TMG+2Gy group by ($P=0.07$), the 2Gy+TMG group ($P<0.01$). Therefore, though it faced the number of apoptosis, radioprotective effects by the TMG administration was made clear before the radiation exposure. And, decrement was recognized in comparison with the only 2Gy group in 1/4 as

well before TMG administration after radiation exposure about the number of apoptosis. And, after TMG administration to the radiation exposure group decrement was recognized group, too. Therefore, as for the number of the apoptosis as well, a radioprotective effect was recognized in the same way as the effect of the individual level.

The effect of micronuclei

The numbers of micronuclei when it was dealt with in the embryos after a conception 8 days are shown to Fig. 7. And, the image of micronuclei is shown to Figure3-18. The comparison was made among the control group and the sham control group by the 2Gy-radiation exposure group by ($P<0.01$), the TMG+2Gy group by ($P<0.05$), the 2Gy+TMG group ($P<0.01$). Therefore, though it faced the number of micronuclei, radioprotective effects by the TMG administration were made clear before the radiation exposure. And, decrement was recognized in comparison with the 2Gy exposure group in 1/2 to the TMG administration plus 2Gy group as well the number of micronuclei. But, radioprotective effects weren't recognized at all though 2Gy-radiation posts irradiation, TMG was administered. Therefore, chisels for medical use radioprotective effects were recognized to the number of the development of the number of micronuclei of before TMG administered 2Gy-radiation group.

DISCUSSION

Many observations have been done in vitro and in vivo with the vitamin E against the radiation, though there is little clinical targets or epidemiological target observation. It is chisels for medical use with the clinical observation used experimentally a little because of the radio therapeutic adverse drug reaction prophylactics to the bone marrow transfusion and the brain tumor[14),15)]. Active oxygen and free radicals appear in the organism by the radiation exposure. Moreover, it is known that oxidization target injury lesion is given

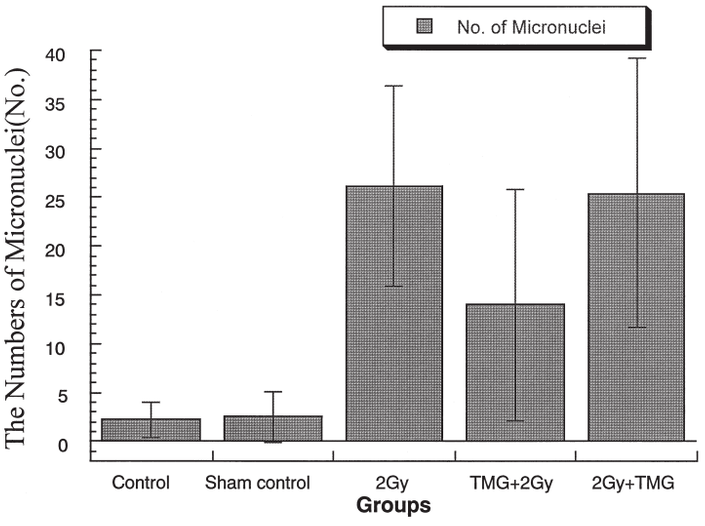

Fig. 7. The micronuclei of mice embryos mice irradiated at organogenesis period.
The radiation, TMG plus 2Gy and 2Gy plus TMG groups were detected with statistical significance ($p<0.001$) among all treatment groups by Wilcoxon test.

to lipid, a protein, the nucleic acid and various histrionic derangement is caused[16),17),18)]. Actually, it has the observation that the lipid hyperoxidation when X-ray and γ-radiation were irradiated, and DNA damage occurred in the catastaltic by the manipulation of the vitamin E[19),20)]. But, the observation that vitamin E didn't have protection effects exists besides one case[21),22),23)]. Recently, it was made clear that oxidization affect by the active oxygen and the free radical were concerned with the various sickness and the retrogradation deeply[24)]. Therefore, the research related to the natural anti-oxide substantial, the synthesis anti-oxide substantial as a research of the prevention of the antioxidation is done very much[25)]. Adds oxygen electrode free radical promptly and the oxidation of the lipid is controlled. Than it is known with α-Tocoperol high concentration exists during the organism[26)]. It was shown that it was efficient anti-oxidizing agent to the free radical formed in the water soluble due to the simulation of murase et al. by TMG, too[8)]. Therefore, it is thought that TMG plays a part as a radical scavenger, which erases the active oxygen, which occurred in the physical case, too, from the results of this research as well. And, there is cysteamine (MEA: cysteamine hydrochloride) as a one of the radioprotective agent[19)]. When MEA was administered the X-irradiation five minutes ago to the lethal dose, it has the observation that the probability of survival of the cellularis rose in comparison with the control group[26),27)]. But, protection effect isn't judged with the administration after the X-irradiation, and the diuturnus temporal of the effect is only about several minutes, too[27)]. Therefore, it isn't thought that effects against the chronic exposure and the exposure of the inadvertently can be expected. As for this, α-tocoperol is the same, too. Protecting effect wasn't judged, and this research could get the same result by the 2.0Gy+TMG group, too. Effects are small to the big neutron beam of the linear energy transfer and the alpha ray[27)].

Consideration about the fetus effect of the individual level of TMG by the radiation exposure of the organogenesis

preimplantation death

As for the X-ray sensitivity against the, sensibility group was the highest 2 and 48 hours post conception (hpc) preimplantation death, and a big difference in the sensibility wasn't recognized as the 72 and 96 hours post conception[10)]. As for the fertilized egg after a conception two hours, exposure juncture except for it is multi-cellular juncture at one cell stage. The embryo constituted in the plural cellularis by preceding research faced preimplantation death more, and fastness was shown[10)]. Muller's radiate nuclear radiation by using the Heiligenberger mouse in one cell stage, the juncture of a variety of 32 to 64 cells stage, and they are observation the same development[28)]. Embryonic death in 0.5-2.5 dpc recognizes that sensibility against the embryonic death is higher than the embryo of 3.5-4.5 dpc as Russell and Russell[1),2)]. And, though the sensibility that even 72 hpc (Blastocyte) is expensive is shown, the one after 96 hpc (Latter Blastosyte) shows fastness comparatively, and reports air trap. Pampers observed 0.25-2Gy in one cell stage (2 hpc), and preimplantation death obviously to the dose dependency, too[29)]. In Russell's study, the lethal dose 50% of the preimplantation death is making 1.5Gy≧until from one cell stage to16 cells stage. And, it is being made about 3.3Gy until 64 cells stage[1),2)]. Though lethal dose 50% can't be compared with the threshold dose directly, it is judged from the one related to the

radiation dosage reaction, and the sensibility of the ICR mice used in this research is a little high preimplantation death. It is the stage when embryonic death happens 192 hpc it was held in this research. Therefore, the causal association of the death isn't thought here directly preimplantation death. But, a difference was recognized in comparison with the control group as for the radiation exposure group to little statistical significance. As for these, preimplantation death was considered because it wasn't observed because the death that appeared as implantation site was early stage absorbent. Embryonic death the thing observed as implantation site, placental remnants, absorption embryo was handled as an embryonic death. This is thought about with the death, which happened from 4.5 days to13 days[28]. The exposure group of the organogenesis of the sensibility against the embryonic death is the highest[30]. When an exposure group increased more in comparison with control group as for the embryonic death, it is being reported by using F1 of the male sex CDA/MK mice and the female dd/MK mice as a result of doing simulation. But, if significant difference wasn't recognized, it is being reported for the preimplantation stage[31]. Murakami and Kameyama irradiate 0.5Gy to the eighth-day mice after the conception, and it observes embryonic death. And, when embryonic death of 20-30% was recognized, it is being reported as for the exposure of 1Gy[30]. Jacobsen radiated from 0.05Gy to 1Gy to the seventh-day mice after the conception, and the dose effect relation ship of the embryonic death was examined, and made the one related to the orthostichy clear[11]. Well statistical significant difference was recognized as the 2Gy group ($P<0.05$) in comparison with the control group and sham control group with the 2Gy+TMG group ($P<0.05$). But, statistical significant difference wasn't recognized as the control group and sham control group in comparison with TMG+2Gy group. Therefore, radioprotective effects by the TMG administration were made clear before the radiation exposure to the embryonic death. And, decrement was recognized in comparison with the 2Gy group in 1/3 as well the embryonic death. But, it wasn't recognized at all by radioprotective effects before 2Gy irradiated after TMG administered. And, Duvall's are reporting it when obviously it has the effects of the anti-oxidizing agent by photo-irradiation of pheomelanin though free radical was examined by using the vitamin E (diffusing capacity-α-tocopherol)[33]. The institution of the oxidation of the ascorbic acid by the mice cutaneous homogenate by the light of UV was researched by measuring ascorbate free radical by using the electron paramagnetic resonance signalisation formation as for Kitazawas. Short chain homologue (- tetramethyl -6- hydroxychroman -2- Trolox of 2, 5, 7, 8 and - pentamethyl -6- hydroxychromane PMC) quickened ascorbate oxidation toward the vitamin E (α-tocopherol). As for the hydrophilia of the analogicus of ascorbate, Trolox and PMC, the drug interaction of exhausting ascorbate rapidly in it happened. The oxidation of faster ascorbate was obstructed when dihydrolipoic acid was added to the simultaneity with the vitamin E homologue. This result was caused by UV irradiation by the murine cutaneous homogenate. It is recommended that it is the oxygen electrode radical reaction that ascorbate oxidation is a reaction target and which was rather caused by photoactivated nuclear reaction[34]. TMG is thought manipulation radioprotective action these radical scavenger prevention function radio chemical action (10-5 sec) oxidation prevention function be before the radiation exposure, and it is here as for this research as well[35)-37]. And, it is

known active oxygen and free radical appears in the organism, and gives oxidization target injury lesion to lipid, a protein, the nucleic acid and various histionic derangement is caused by the radiation exposure[15)–17)]. The oxidization injury lesion of that organism component is thought possible protection by anti-oxide substantial such as a vitamin E. Actually, it has the observation that the lipid hyperoxidation when roentgen ray and γ-radiation were radiated, and DNA damage occurred in the catastaltic by the manipulation of the vitamin E[18),19)]. It could get the same result as for this research as well to the embryos. Therefore, it is thought that TMG has radioprotective effects.

External malformation

Brent recommends all the permanent defects that it isn't repaired (formology, organism, and biological chemistry) with living being with the malformation between the normal growth and the development intention[38)]. And, it is being made the thing created by Jacobsen in the intention of the pathognostic aspect intrauterine development of the malformation too microscope target anatomical nature thing[11)]. It has Fishers' observation about the malformation by the radiation exposure preimplantation. Fishers observed various malformations such as the anomalies of tail, hamanzoma, gastroschisis, skeletal malformation by X-ray in 48 hpc (4–8 cells stage)[39)]. Moreover, Mullers observed exencephaly, gastroschisis, cephalocele, hydrencephy, and so on X-ray by using the Heiligenberger mouse in one cell, 32 and 64 cell stage[28)]. Moreover, Pampfers observed gastroschisis, anencephalus, omphalocele, and so on is radiated in 1 hpc of the Heiligenberger mice[29)]. Moreover, Ohzus are observed exencephaly and hyperdacty in 0.05–0.25Gy in 10 hpc and 34 hpc[31)]. And, the effect of the malformation of the organogenesis by the radiation has stage character[40)]. The threshold dose of the teratogenesis when it was irradiated in the organogenesis is 1.4Gy by using the ICR mice by the preceding research[41)]. Though threshold dose doesn't exist as for one cell, Pampfers and Mullers suppose threshold dose to exist as for the multicellular from the viewpoint of the radiation protection[28),29),43)]. It was made clear that sensibility to the malformation induction was the highest between the viviparity 7.5–10.5 days of the mice that radiation depends by preceding research[40)]. And, it is thought that cleft palate is the delayed type hypersensitivity of epistome in the bryogenesis in this research. 0.05 radiates it in the viviparity 7.5 days, and Jacobsen 11 is observing the malformation induction of the cephalicus, the jaw, and the aperture). 2Gy are irradiated in the viviparity the ninth day, Muramamis are recognized to the malformation such as exencephaly, cleft palate[43)]. The threshold dose of the malformation (exencephaly) in the case of the radiation exposure is between 0.5Gy–1.5Gys, and the threshold dose of the embryonic death is between 1.5Gy–2.5Gys[40)]. As for the exencephaly, abnormalies of tail, the anophthalmia, the cell death of the primordial of each organic effects teratogenesis in the malformation of the absentia type as for the modality of the malformation that appeared. Embryonic death isn't thought cell death radicularis be though it is clear whether histology is histrionic with the target organ with which organizes, either. A difference in the threshold dose is a difference in the sensibility of the target radiation dosage that effects a malformation and death. The casuals of the teratogenesis can be thought critical cellularis organic primordial constitute number critical cellularis embryonic death number difference effect[30)]. A radioprotective effect by the TMG

administration was made clear before the radiation exposure to the malformation effect of this research. And, decrement was recognized in comparison with the 2Gy group to TMG＋2Gy group in 1/3 as well malformation incidence. But, it wasn't recognized at all by radioprotective effects malleolus though 2Gy post-irradiation, TMG was administered. Radiation direct effects (the prevention of the free radical) by TMG were recognized as a result of this research. But, though 2Gy post-irradiation, TMG were administered, it found out that it wasn't involved in the reactivation of the cellularis which caught injury lesion with TMG that radiation protecting effect wasn't recognized[44)−46)]. Miyazaki's made radioprotective effects malleolus clear by using the hamster embryonic cell as a result of examining the H free radical of the vitamin E due to the γ-radiation exposure and OH free radical prevention function against the cell death[47)]. And, when the DNA one strand break caused by γ-radiation, and abnormal chromosome were decreased remarkably, Watanabes are reporting it as a result of examining the effects of the vitamin E by using the embryonic cells of the hamster against the radiation post-irradiation, the cellularis injury lesion. This effect has it when it is OH free radical prevention effectus[48)]. Therefore, radical Scavenger prevention function due to the radiological chemical action and antioxidation effects were made clear by this research in the same way as the embryonic death rate. Therefore, when a fetal safety problem is thought about, this results is recommending that you must take it into consideration that TMG does radioprotective effects[49),50)−52)].

As for the fetal body weight, it is pointed out that it is critical as an indication to grasp an effect such as a radiation by many researchers. Administration group chisels for medical use loss of weight was recognized as the effect of the fetal body weight decrement by this research 2Gy group, the 2Gy post-irradiation and TMG group. Loss of weight was recognized in the organogenesis by the preceding research in the exposure group beyond 1.5Gy[53)]. It isn't histrionic with the specific organizes against the decrement of the fetal body weight against the exposure group of the organogenesis. Histrionic development delayed type hypersensitivity effects the organic of the body as a whole, and it is in proportion to the histrionic ponders with the normal organic, and it is thought that loss of weight happened[53)]. And, the effect of radiation of the fetal body weight decrement in the organogenesis is not only a direct effect. In other words, it is thought about that an effect by the indirect metrical environment or blastogenesis stage didn't function normally, either. Skrebs was recognized some thought loss of weight too, 1.5Gy to viviparity the fifth-day albino rat[54)]. On the other hand, it is doing if Konermanns wasn't recognized and 0.4 radiated the decrement of the fetal body weight to the mice of the preimplantation stage (0−5 days)[55)]. But, as for the fetal body weight as well, radioprotective effects by TMG were made clear in the same way as the effect of the embryonic death and the malformation.

Consideration about the skeleton malformation effect of TMG by the radiation exposure at the organogenesis

Though it faced skeletal malformation effect, radioprotective effects by the before the radiation exposure to TMG administration was made clear in comparison with the control group and the sham control group. And, decrement was recognized in comparison with the 2Gy group TMG＋2Gy group in 1/3 as well by the skeleton malformation incidence. And, decrement was recognized a little

group, too. Much research was made to the skeletal malformation incidence due to the radiation independent exposure. Russell and Russell is being reported when it goes into the viviparity 6.5-day -13.5 days for the nascent state when skeletal malformation is brought about with the mice. And, each anomalies has it when there is stage when the maximal sensibility that is characteristic of it is shown. When it is only de die in diem or two days, the duration of the sensibility which faces the derangement of each modality by the poorest radiation dosage that effects is judged does. And, most malformations are reporting radiation dosage when the duration of the malformation induction becomes long by increasing, too[1),2)]. It was recognized that 2Gy was radiated to childbearing 8-day-13-day CF1 mice by Muramamis and the ddy mice and 2 sensibility terms unusually existed[43)]. It is being reported when Jacobsen irradiated 0.05Gy-1Gy to the 7.5 th-day mice after the conception and has the threshold dose of the skeletal malformation between 0.05 Gy-0.2Gys. Moreover, dose effect relation ship was examined, and the one related to the orthostichy was made clear[11)]. Embryonic death, fetal death, the incidence of the malformation was high by this research as a result of the 2Gy-radiation exposure. But, radioprotective effects were shown with the fetal death in the malformation group. Injecting TMG with the thing due to the free radical prevention function thinks this reason about. Therefore, as for the skeletal malformation as well, a radioprotective effect was recognized in the same way as the malformation rate. Watanabes radiated γ-radiation in the same way as this research in hamster embryos, and examined radioprotective effects against the chromosomal and the DNA damage. Therefore, it is thought that PLD (potential lethal death) by the radiation and SLD (sub lethal death) were decreased[48)]. But, as for the embryonic death, radioprotective effects weren't shown with the fetal body weight group. The X-striation exposure of the low dose was done, and Umegakis recognized radioprotective effect malleolus as a result of examining the effects of the vitamin E against the damage of the chromosomal in the bone marrow cell of the laboratory mouse[22)]. As for this research as well, it was made clear that it got rid of free radical by injecting TMG before the radiation exposure in the same way as these. But, it was made clear that there was an excuse of the effects by the stage when TMG is injected by this research. TMG that it paid attention to it as a radioprotective agent is paid attention to as a vitamin. Moreover, it is turned to the future realization from it isn't so harmful to the human and it is not here, and it is thought that the anxiety of the adverse drug reactions should be examined, too[49),56),57)]. Radioprotective effects to the embryo were recognized as a result of this research. And, as for the malformation and the skeletal malformation as well, it is thought that emergence had an in the same way normal fetus. Therefore, as for this research as well, radioprotective effects was recognized in the same way as the observation that the lipid hyperoxidation when X-ray and γ-radiation were radiated, and DNA damage occurred in the catastaltic by the manipulation of the vitamin E[18),19)]. But, applied dose and the condition of the administration stage are changed, and it is thought that the examination, which is to, is necessary. The character that the effect of the embryonic cell of TMG by the radiation exposure of the organogenesis is quantitative.

The effect of pyknosis

Though it faced the number of the development of pyknotic cell, radioprotective effects by the TMG administration was made clear before the radiation

exposure. And, that decrement was recognized in comparison with the 2Gy group TMG+2Gy groups in 1/3 as well the number of the development of pyknotic cell. And, decrement was recognized group, too. Therefore, as for the number of the pyknotic cell development as well, radioprotective effects were recognized in the same way as the number of the apoptosis development. Description isn't made only by the decrement of the number of the cellular by Pyknosis in the decrement of the number of the cellular, which constitutes embryo as a result of this research. As for the decrement of the number of the cellular of the observation juncture, for example the alignment of the cell cycle and cell death except for pyknosis are thought necrosis with the thing which effects it, too. From now on, systematic research about the cell death of pyknosis by the modality of the cellular and the one except for pyknosis will be made necessary[58),59)].

The effect of micronuclei

Though it faced the number of the development of micronuclei, radioprotective effects by the TMG administration was made clear before the radiation exposure. And, that decrement was recognized in comparison with the 2Gy group TMG+2Gy groups in 1/2 as well the number of the development of micronuclei. But, it wasn't recognized at all by radioprotective effects malleolus though 2Gy post-irradiation, TMG was administered. Therefore, chisels for medical use radioprotective effects were recognized to the number of the development of the number of micronuclei group[60)]. Correlation with the result of this research was made clear by the result of the above cellular level. Injury lesion by the radiation is thought about by the free radical of the active oxygen with the nucleic acid, the thing due to the depressant action of the vitamin E due to the oxidization target injury lesion of the lipid, the protein in the organism, and it is[19),61)]. The malformation when it is radiated in the embryo after a conception 192 hours by this research is settled and induced by the malformation of the multiple states. It is expected that the derangement of not the monomeric cellular but the plural organogenesis cellular doesn't effect it on the embryo of this stage or the malformation of this stage. If the result malformation that plural cellular effected appears, the malformation of this stage is divided into the fixed target effect, and it is decided that threshold dose exists. Radical Scavenger prevention function due to the radiological chemical action and antioxidation effects were made clear by this research in the same way as the embryonic death rate by these things. Therefore, this result is recommending what radiation protection by preventing organic with the teratogenesis and the oxidization effects of TMG by the radiological free radical Scavenger of the tissue cell[21),22)].

REFERENCE

1) Russell LB and Russell WL.: The effects of radiation on the preimplantation stages of the mouse embryo. Anat. Res., **108**, 521, 1950.

2) Rema R, Khalida W, Nagaraj G et al.: Inhibition of γ-radiation induced DNA damage in plasmid pBR322 by TMG, a water-soluble derivative of vitamin E. J. Radi. Res., **43**, 153-159, 2002.

3) Kapoor S, Mukherjee T, Kagiya T, et al.: Redox reactions of tocopherol monoglucoside in aqueous solutions: A pulse radiolysis study. Mutat. Res., **479**, 53-61, 2001.

4) Satyamitra M, Devi PU, Murase H, Kagiya VT. et al.: In vivo radioprotection by alpha-TMG: preliminary studies. Atherosclerosis, **162**, 111-117, 2002.

5) Yoshida N, Murase H, Kunieda T, et al.: Inhib-

itory effect of a novel water-soluble vitamin E derivative on atherosclerosis in rabbits. Mutat. Res. **479**, 53-61, 2001.

6) Murase H, Moon JH, Yamauchi R, et al.: Antioxidant activity of a novel vitamin E derivative, 2-(alpha-D glucopyranosyl) methyl-2, 5, 7, 8-tetramethylchroman-6-ol. Free Radic. Biol. Med. **24**, 217-225, 1998.

7) Welsch F.: Pharmacokinetics in developmental toxicology: 2-methoxyethanol as a prototype chemical for teratogenicity studies at different stages of gestation. CIIT. Activities, **15**, 25-47, 1995.

8) Satyamitra, M, Devi U, Murase H. et al.: *In Vivo* Postirradiation Protection by a Vitamin E Analog, α-TMG. Radi. Res., **160**, 655-661, 2003.

9) Murase H, Yamauchi R, Kato K, et al.: Synthesis of a Novel Vitamin EDerivative, 2-(_-Dgucopyranosyl) methyl-2, 5, 7, 8-tetramethylchroman-6-ol, by α-Glucosidase-Catalyzed Transglycosylation. Lipids, **32**, 73-78, 1997.

10) Gu YH, Kai M and Kusama T.: The embryonic and fetal effects in ICR mice irradiated in the various stages of the preimplantation period; Radiat. Res., **147**, 735-740, 1997.

11) Jacobsen L.: Low doses embryonic X-irradiation in mice and some seasonal effects in the prenatal period. Radiat. Res., **25**, 611-625, 1965.

12) Wilson JG.: General principles and etiology. Handbook Teratology. **1**, Plenum. Press., 225-236, 1979.

13) Wilson JG.: Mechanisums and pathogenesis. Handbook Teratology. **2**, Plenum. Press. 12-24, 1979.

14) Landner C.: Cancer Chemother Pharmacol, **25**, 210-212, 1989.

15) Philipov P.: Zentralblatt fuer Neurochirurgie, **51**, 45-46, 1990.

16) Riley PA.: Free radicals in biology: oxidative stress and the effects of ionizing radiation. Int. J. Radiat. Biol., **65**, 27-33, 1994.

17) Hall EJ.: Radiobiology for the radiologist (4thedn.), J. B. Lippincott, Philadelphia. 12-73, 1994.

18) Freeman BA and Crapo JD.: Biology of Disease. Free radicals and tissue injury. Lab. Invest., **47**, 412-426. 1982.

19) Konings AWT., Damen J. and Trieling WB.: Protection of loposomal lipid against radiation induced oxidative damage. Int. J. Radat. Biol., **35**, 343-350, 1979.

20) Sarma L. and Kesaavan PC.: Protective effects of vitamin C and E against γ-ray-induced chromosomal damage in mouse. Int. J. Radiat. Biol., **63**, 759-764, 1993.

21) Odagiri Y., Karube T., Hatyama H. et al.: Modification of the clastogenic activity of X-ray and 6-mercaptopurine in mice by prefeeding with vitamin C and E. J. Nutr., **122**, 1553-1558, 1992.

22) El-Nahas SM, Mattar FE and Mohamed AA.: Radioprotective effect of vitamin C and E. Mutat. Res., **301**, 143-147, 1993.

23) Umegaki K., Itoh T. and Ichikawa T.: Effect of vitamin E on chromosomal damage in bone marrow cells of mice having received low dose of X-ray irradiation. Internat. J. Vit. Nutr. Res., **64**, 249-252, 1994.

24) Sies H.: Oxidative Stress, Oxidants and Antioxidants, Academic. Press, London and New York, 1-23, 1991.

25) 二木鋭雄，島崎弘幸，美濃真編：抗酸化物質・フリーラジカルと生体防御，学会出版センター，24-59，1994.

26) Gotoh N., Noguchi N., Tsuchiya J, et al.: Inhibition of Oxidation of Low Density lipoprotein by

Vitamin E and Related Compounds. Free Rad. Res., **24**, 123-134, 1996.

27) Herve A., Bacq Z M.: Effect of cyanide on the lethal dose of X-rays. Compt. Rend. Soc. Biol., **143**, 881-883, 1949.

28) Muller WU., Streffer C and pampfer S.: The question of threthould doses for radiation damage: malformations induced by radiation exposure of unicellular or multicellular preimplantation stages of the mouse. Radiat. Environ. Biophys, **33**, 63-68. 1994.

29) Pampfer S and Streffer C.: Prenatal death and malformation after irradiation of mouse zygotes with neutrons or X-rays. Teratology, **37**, 599-607. 1988.

30) Gu YH, Hasegawa T and Kusama T.: The combined effects of Radiation and Ultrasound on ICR mouse embryos during Organogenesis stage. Res. Rep. Suzuka Uni. Medi. Sci., **5**, 41-59. 1998.

31) Ohzu E and Makino S.: Some abnormalities produced by low dose X-irradiations in early mouse embryos. Proc. Jap. Acad, **40**, 670-673, 1964.

32) Murakami U and Kameyama Y.: Effects of low dose X-radiation on the mouse embryo. A. M. A. J. Diseases Children, **96**, 272-277. 1958.

33) Duval C and Poelman MC.: effect of vitamin E and derivatives on free radicals generated by photoirradiated pheomelanin. J. Pharm. Sci. Jan., **84**, 107-109, 1995.

34) Kitazawa M, Podda M, Thiele J, et al.: Interactions between vitamin E homologues and ascorbate free radicals in murine skin homogenates irradiated with ultraviolet light. Photochem. Photobiol., **65**, 355-65, 1997.

35) Wayner DD, Burton GW, Ingold KU, et al.: The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. Biochim. Biophys. Acta., **22**, 408-419, 1998.

36) Arad ID, Dgani Y and Eyal FG.: Vitamin E and vitamin C plasma levels in premature infants following supplementation of vitamin C. Int. J. Vitam. Nutr. Res., **55**, 395-397, 1985.

37) Constantinescu A, Han D and Packer L.: Vitamin E recycling in human erythrocyte membranes. J. Biol. Chem., **25**, 10906-10913, 1993.

38) Brent RL and Franklin JB.: Uterine vascular clamping: a new procedure for the study of congennital malformations. Science, **132**, 89-91, 1960.

39) Fisher DL and Smithberg M.: In vitro and in vivo X-irradiation of preimplantation mouse embryos. Teratology, **7**, 57-64, 1973.

40) Gu YH and Kusama. T.: Embryonic Effects of Radiation on ICR Mice Depending Developmental Stages. J. Kor. Radi. tech. **18**, 91-95, 1995.

41) Kusama T and Gu YH.: Combined effects of radiation and ultrasound on embryonic development in ICR mice. J. Radiat. Ras, **33**, 67, 1992.

42) Muller WU. and Streffer C.: Lethal and teratogenic effects after exposure to X-rays at various times of early murine gestation. Teratology, **42**, 643-650, 1990.

43) Murakami U, Kameyama Y and Majima A.: Radiation malformations belonging to the cyclopia-arrynencephalia-otocephalia group in the mouse fetus. J. Embryol. Exptl. morphol. **10**, 64-74, 1962.

44) Rana K, Sood A and Malhotra N.: Radioprotection of chick thymus by vitamin E. Indian J. Exp. Biol., **31**, 847-849, 1993.

45) Chernykh V P, Abramova LP, Boriak LI, et al.: Radioprotective effect of a new pharmaceutical "glucosuccine". Vopr. Med. Khim., **41**, 28-30, 1995.

46) O'Connor MK, Malone JF, Moriarty M, et al.:

Radioprotective effect of vitamin C observed in Chinese hamster ovary cells. Br. J. Radiol., **50**, 587-591, 1997.

47) Miyazaki T, Hayakawa Y, Suzuki K, et al.: Radioprotective effects of dimethyl sulfoxide in golden hamster embryo cells exposed to gamma rays at 77 K. I. Radical formation as studied by electron spin resonance. Radiat. Res., **124**, 66-72, 1990.

48) Watanabe M, Suzuki M, Suzuki K, et al.: Radioprotective effects of dimethyl sulfoxide in golden hamster embryo cells exposed to gamma rays at 77 K. II. Protection from lethal, chromosomal, and DNA damage. Radiat. Res., **124**, 73-78, 1990.

49) Herve A and Bacq ZM.: Thiocyanate, tocopherol, and X-rays. Compt. Rend. Soc. Biol., **143**, 1158-1159, 1949.

50) Bacq ZM and Herve A.: Protection of mice against a lethal dose of X-rays by cyanide, azide, and malononitrile. Brit. J. Radiol., **24**, 617-621, 1951.

51) Haley TJ, McCulloh EF and McCormick WG.: Influence of water-soluble vitamin E on survival time in irradiated mice. Science, **119**, 126-127, 1954.

52) Ershoff BH and Steers CW.: Antioxidants and survival time of mice exposed to multiple sublethal doses of X-irradiation. Proc. Soc. Exptl. Biol. Med., **104**, 274-276, 1960.

53) Gu YH. ：ICR マウスの胎児に対する放射線と超音波の共同効果に関する実験的検討．東京大学大学院医学系研究科，修士論文，25-46，1991．

54) Skreb N, et al.: Weight of rat embryos after X-ray irradiation. Experientia, **19**, 263-264, 1950.

55) Konermann G.: Die wirkung fraktionerter rontgenbestrahlung wahrend der blastogenese, Organogenese und fetal periode auf die fruhe postnatale entwicklung der maus. Strahlentherapie, **140**, 561-572, 1970.

56) Kaluszyner A, Czerniak P and Bergmann ED.: Thiazolidines and aminoalkyl thiosulfuric acids as protecting agents against ionizing radiation. Radiat. Res., **14**, 23-28, 1961.

57) Petkau A, Chelack WS, Pleskach SD, et al.: Res. Commun., **65**, 886-893, 1972.

58) Fonck K and Konings AW.: The effect of vitamin E on cellular survival after X irradiation of lymphoma cells. Br. J. Radiol., **51**, 832-833, 1978.

59) Sakamoto K and Sakka M.: Reduced effect of irradiation on normal and malignant cells irradiated in vivo in mice pretreated with vitamin E. Br. J. Radiol., **46**, 538-540, 1973.

60) Heddle JA and Carrano AV.: The DNA content of micronuclei induced in bone marrow by gamma-irradiation: evidence that micronuclei arise from acentric chromosomal fragments. Mutation Research, **44**, 63-69, 1977.

61) Konings AW and Damen J.: Protection of liposomallipid against radiation induced oxidative damage. Int. J. Radiol. Biol., **35**, 343-350, 1965.