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# ENHANCEMENT OF BIO-PROTECTIVE FUNCTIONS BY LOW DOSE/DOSE-RATE RADIATION

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□ Effects of low-dose-rate gamma-irradiation on the process of tumorigenesis were investigated in mice treated with a carcinogenic agent or irradiated with high dose X-rays at a high dose rate. A prolonged gamma irradiation at approximately 1 mGy/hr suppressed the appearance of skin tumors induced by methylcholanthrene and delayed the appearance of radiation-induced thymic lymphomas in C57BL/6 mice. We also investigated the effects of low-dose-rate irradiation on disease model mice. In Type II diabetic C57BL/KsJ-db/db (db) mice, the urine glucose level was improved in some of the mice irradiated at 0.70 mGy/hr, but not in non-irradiated control mice. In MRL-lpr/lpr (lpr) mice with severe autoimmune diseases, immunological status was kept better in the mice irradiated at 0.35 or 1.2 mGy/hr. The incidence of a number of symptoms, including lymphadenopathy, splenomegaly and proteinuria, was suppressed by the irradiation. Furthermore, in both of the strains, the low-dose-rate irradiation prolonged the life span of the irradiated mice.

## I. INTRODUCTION

Low doses of ionizing radiation stimulate various biological functions: anti-oxidative capacity[1], DNA repair capability[2], apoptosis[3, 4], and immune functions[5, 6]. Each of these functions may work in a suppressive manner in the process of carcinogenesis, which would be initiated with DNA damage induced directly by radiation or through reactive oxygen species production. We have previously demonstrated that a low-dose-rate irradiation at around 1 mGy/hr suppressed the incidence of methylcholanthrene-induced skin tumors in ICR mice[7]. We also found some of biological protective functions, including anti-oxidative capacity and immune functions, were enhanced. To examine the possibility that the augmented protective capacity may have some other effects in addition to the tumor suppression, we investigated the effects of low dose irradiation on disease model mice.

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**FIGURE 1.** Long-term low-dose-rate irradiation facility

## II. IRRADIATION FACILITIES

Irradiation with low-dose-rate gamma rays was carried out in a clean irradiation room (Figure 1) equipped with a $^{137}$Cs gamma-ray source (370 GBq). The dose rates at 5, 7 and 10 m from the source were 1.2, 0.70 and 0.35 mGy/hr, respectively. The irradiation was continued, except for 1 hr intermissions in the morning on weekdays for care and examination of the mice. The design of the irradiation facility and the details of dosimetry with the ionization chamber and glass dosimeter have been described elsewhere[8].

## III. EFFECTS OF LOW DOSE/DOSE-RATE IRRADIATION ON THE PROCESS OF TUMORIGENESIS

### Chemically Induced Skin Tumors

Groups of thirty-five 5-week old female C57BL/6N mice were irradiated with $^{137}$Cs gamma rays at a dose rate of 0.35, 0.70 or 1.2 mGy/hr for 5 weeks. Then, to induce skin tumors, the mice were subcutaneously injected in the groin with 20-methylcholanthrene (MC), which had been dissolved in olive oil, with each mouse receiving 0.1 ml[9]. The dose of MC was 0.05 mg/mouse. After the MC injection, irradiation was continued at the same dose rate. All animals were given food and water *ad libitum* following the guidelines for animal experiments at CRIEPI.

The first tumor appeared 53 days after the MC injection in non-irradiated control group. In the 0.35 mGy/hr group, the first tumors appeared 75 days after the MC injection. In the 0.70 mGy/hr group, it appeared 115 days after the injection. And in the 1.2 mGy/hr group, it appeared 108 days after the injection. Cumulative tumor incidences, 200 days after the MC injection, are shown in Figure 2.

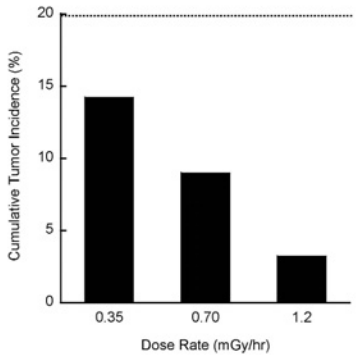


**FIGURE 2.** Cumulative tumor incidence in MC-injected mice irradiated at different dose rates. The dotted line is the cumulative tumor incidence in the non-irradiated control group.

### Radiation-Induced Thymic Lymphomas

Thymic lymphomas were induced in female C57BL/6N mice by four weekly X-irradiations of 1.8 Gy each. A group of 20 female mice, 5 weeks old, were exposed to a low dose rate of 1.2 mGy/hr for 5 weeks, and then they were subjected to the repeated high dose irradiation at a high dose rate. Another group received only the fractionated high dose irradiations. The first mouse death of thymic lymphoma occurred 89 days after the last irradiation in the group that received only the repeated high dose irradiations, while 110 days elapsed before the first death in the group that received the repeated irradiations combined with the low-dose-rate irradiation. The low-dose-rate irradiation appears to have delayed the timing of the appearance of the lymphomas (Table 1).

## IV. EFFECTS OF LOW-DOSE-RATE IRRADIATION ON DISEASE MODEL MICE

### Type II Diabetes

A group of 12 female 10-week old C57BL/KsJ-*db/db* model mice with Type II diabetes mellitus[10] were irradiated for life at 0.70 mGy/hr. The urine glucose levels of all of the mice were strongly positive at the beginning of the irradiation. In the irradiated group, a decrease in the glucose level was observed in three mice, one in the 35$^{th}$ week, another in the 52$^{nd}$ week and the third in the 80$^{th}$ week. No recovery from the diabetes was observed in the 12 mice of non-irradiated control group.

There was no systematic change of body weight or consumption of food and drinking water between the irradiated group and the non-irradiated group or between the recovered mice and the non-recovered mice.

**TABLE 1.** Effects of low dose irradiation on the appearance of radiation-induced thymic lymphomas

| | THYMIC LYMPHOMAS (%) | |
|---|---|---|
| Days after 1.8 Gy irradiations | Repeated high dose irradiations only | Low-dose-rate irradiation before repeated irradiations |
| 90 | 5 | 0 |
| 100 | 20 | 0 |
| 120 | 20 | 10 |
| 140 | 45 | 25 |
| 160 | 70 | 55 |

As shown in Table 2, survival was better in the irradiated group. A marked difference was also observed in the appearance of the coat hair, skin and tail. The irradiated group was in much better condition.

Mortality was delayed and the healthy appearance was prolonged in the irradiated mice by about 20-30 weeks compared with the control mice. These results suggest that the low dose irradiation modified the condition of the diabetic mice, leading not only to recovery from diabetes, but also to suppression of the aging process.

### Severe Autoimmune Disease

MRL-*lpr/lpr* mice[11] have a deletion of an apoptosis-regulating gene, *Fas*, that causes abnormal proliferation activity of lymphocytes, leading to severe total-body lymphadenopathy, splenomegaly and many autoimmune diseases. Groups of 12 female MRL-*lpr/lpr* mice, 7 weeks old, were irradiated with $^{137}$Cs gamma rays at 0.35 or 1.2 mGy/hr for 5 weeks. Then, they were kept in an animal care room without further irradiation. Table 3 summarizes the effects of low-dose-rate irradiation on the survival of the mice. The life span of MRL-*lpr/lpr* mice was markedly prolonged in a dose-rate dependent manner. Some of the symptoms typical in the mice with autoimmune diseases, including lymphadenopathy and proteinuria, were also suppressed[12].

**TABLE 2.** Effects of Low-Dose-Rate Irradiation on Survival of C57BL/KsJ-*db/db* Mice

| | SURVIVAL (%) | |
|---|---|---|
| Age (weeks) | Control | Irradiated |
| 30 | 100 | 100 |
| 60 | 75 | 100 |
| 90 | 42 | 67 |
| 120 | 0 | 33 |

**TABLE 3.** Effects of low-dose-rate irradiation on survival of MRL-*lpr/lpr* mice

| | SURVIVAL (%) | | |
|---|---|---|---|
| Age (days) | 0 (mGy/hr) | 0.35 (mGy/hr) | 1.2 (mGy/hr) |
| 100 | 100 | 100 | 100 |
| 125 | 67 | 100 | 100 |
| 150 | 0 | 83 | 100 |
| 175 | 0 | 25 | 100 |
| 200 | 0 | 17 | 83 |

A cytofluorographic analysis revealed the improvement of the immunological status in the irradiated mice as the decrease in $CD3^+$ $B220^+$ cells, which attack their own organs and tissues, the decrease in $B220^+$ $CD40^+$ cells, a representative cell population in autoimmune diseases, and the increase in $CD8^+$ T cells, which play important roles in immune activity (Table 4).

## V. DISCUSSION

In the present study, suppression of tumorigenic processes was demonstrated (Figure 2, Table I). We have observed some increase in immune functions and tumor cell rejection capability in mice irradiated with 1.2 mGy/hr for several weeks (manuscript in preparation). Therefore, the enhancement of immune functions is presumably involved in the tumor suppression, although the contribution of other protective functions is not excluded. The significant involvement of immune functions was also demonstrated in MRL-*lpr/lpr* mice (Table IV).

The life span prolongation seen in the diabetic mice may not be directly correlated with the cure of diabetes, because the longer life span was seen not only in those mice showing recovery, but also in non-recovered mice. The low-dose-rate irradiation might have affected the process of aging. In the process of ageing, oxidative stress are believed to be involved.[13] The increase in the antioxidative capacity in irradiated mice[1, 14] might explain the anti-ageing effect of low-dose-rate irradiation.

**TABLE 4.** Effects of low-dose-rate irradiation on immune cell populations in MRL-*lpr/lpr* mice

| | FRACTION OF CELL POPULATION (%) | | |
|---|---|---|---|
| Cell Population | 0 (mGy/hr) | 0.35(mGy/hr) | 1.2(mGy/hr) |
| $CD3^+B220^+$ | 42 | 36 | 24 |
| $B220^+CD40^+$ | 13 | 10 | 7.6 |
| $CD8^+$ | 3.1 | 5.6 | 5.8 |

Although their mechanisms are to be elucidated, the phenomena described in the present report should have a significant impact, on one hand, on the basic assumption in the radiological protection, which claims that radiation is harmful no matter how low the dose is. On the other hand, they would be a trigger to explore the possibility of clinical application of low dose/dose rate radiation.

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