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Protection of Survival and Hematopoiesis in Irradiated Mice by 5-Fluorouracil

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The effects of whole-body irradiation on survival and the hematopoietic system were studied in mice treated with 5-fluorouracil (5-FU). Animals (ddY-SLC male mice, 8–10 weeks old) were injected with 5-FU (i.p.) as a single dose (150 mg/kg) at various times before or after irradiation with X-rays at graded doses (4.8 to 7.6 Gy). The treatment of mice with 5-FU 5 days before irradiation was the most effective for the reduction of radiation lethality, having a radioprotective effect. The dose reduction factor (DRF) was 1.24. However, treatment with 5-FU at 1 day and 2 hours before, or at various times after irradiation significantly increased the radiation lethality compared to the untreated controls, creating a radiosensitizing effect. The decrease or the increase of radiation lethality exhibited by 5-FU was similar to the radiation-dose relationship pattern shown by endogenous and exogenous CFU-S. The pattern of change of thrombocyte counts in the circulating blood after irradiation was greatly modified by pretreatment with 5-FU 5 days before irradiation, effectively lessening the radiation-induced depression. In contrast, the post-irradiation patterns of leukocyte and erythrocyte variation did not show any significant change due to pretreatment with 5-FU.

Key words: Survival — CFU-S — 5-Fluorouracil — X-Irradiation — Mice

5-Fluorouracil (5-FU) has been used as a selective agent for killing proliferating cell fractions in hematopoietic stem cell populations.[1–3] This agent is more widely known as a drug used in cancer chemotherapy.[4] There is evidence that hematopoietic stem cells surviving treatment with 5-FU are more primitive and have a greater ability to generate spleen colony-forming units (CFU-S), granulocyte-macrophage colony-forming cells (GM-CFC) and megakaryocyte colony-forming cells (Meg-CFC) than those of untreated mice.[3, 5–10] These results might allow us to test the radiation response of the primitive hematopoietic stem cells in mice treated with 5-FU. In our previous studies, the effects of 5-FU on hematopoietic stem cells in the femur and spleen were examined in normal and irradiated mice. Those unpublished data showed enhanced post-irradiation recovery of CFU-S in mice pretreated with 5-FU and the variation in the radiosensitivity of CFU-S populations after 5-FU treatment. There appeared to be a relation between the survival of irradiated mice and the number or radiosensitivity of the CFU-S population in mice treated with 5-FU.

The present studies were undertaken to investigate the modifying effects of 5-FU on survival, endogenous and exogenous CFU-S and circulating blood cells in X-irradiated mice.

MATERIALS AND METHODS

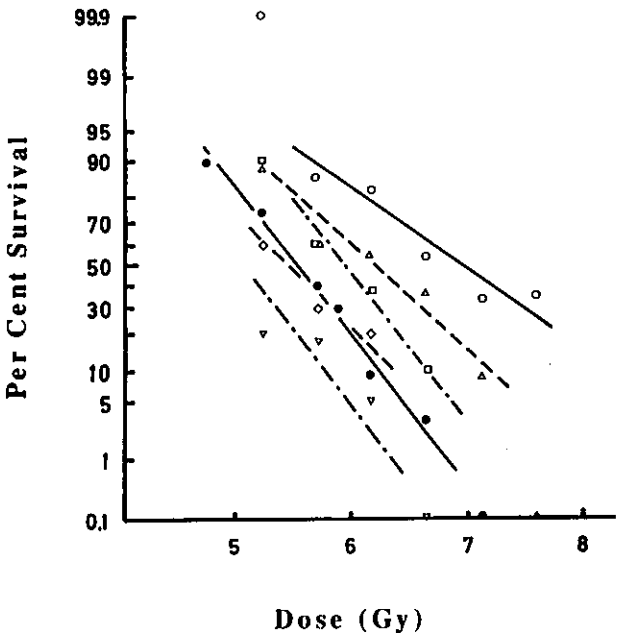
Animals Male ddY-SLC mice of a closed colony stock, 8–10 weeks old and weighing 30–36 g, were used in all experiments. The mice were housed individually in small rooms, separated from each other, and fed with laboratory chow and tap water acidified with 0.1 *N* HCl *ad libitum*. In survival experiments, 20–30 mice per experimental group were used, and some of the experiments were repeated 2–3 times. The survival was determined by checking the number of surviving animals daily in each group during a 30-day post-irradiation period.

5-FU treatment 5-FU (Wako Pure Chemical Industries) at a dose of 150 mg/kg body weight was administered i.p. into mice after dissolution in physiological saline.

Irradiation The irradiation of the mice was performed with X-rays (200 kVp and 20 mA), using 0.5 mm copper and 0.5 mm aluminum filters, at a dose-rate of 0.67–0.69 Gy/min. Several mice were simultaneously given a single whole-body exposure in individual lucite chambers at 58 cm focus aurface distance.

Spleen colony count Assay of endogenous spleen colonies: Mice were irradiated with various doses of X-rays. Nine days after irradiation, the mice were killed for a spleen colony count. The spleens were dissected out, weighed and then fixed with Bouin's solution. The number of visible colonies formed on the surface of the spleen was counted.

Assay of exogenous CFU-S: The exogenous CFU-S technique of Till and McCulloch[11] was somewhat modified and used to estimate the number of pluripotential and transplantable stem cells. Femurs and spleens from 3–8 donor mice were used for the preparation of the cell suspension and were separately prepared in ice-cold TC-199 medium. The cells were injected i.v. into the recipients (10 mice per experimental point), which were irradiated with 7.6 Gy of X-rays 3 to 4 h before the



injection. Nine days after the transplantation of donor cells, a spleen colony count was carried out. Log-linear dose-effect curves, and D_0 and n values were obtained using the following model:

$$S=1-(1-\exp^{-D/D_0})^n$$

Blood cell counting Whole blood drawn from the outer iliac arteries and veins of the mice was collected in tubes containing the anticoagulant EDTA-2K. Leukocytes, erythrocytes and thrombocytes in the circulating blood were counted by using a Sysmex K-1000 30 min after the collection.

Fig. 1. Radiation-dose response curves for the 30-day survival of mice treated with 5-FU at various times before X-irradiation. Each point represents the per cent survival on day 30 and is plotted on a probit scale. —●—, untreated controls (no 5-FU) (30–75 mice per point were used); --◇--, mice with 5-FU treatment at 15 days before irradiation (20 mice per point); -·□-·, 5-FU at 12 days (30 mice per point); —○—, 5-FU at 5 days (30–50 mice per point); --△--, 5-FU at 3 days (25 mice per point); -·▽-·, 5-FU at 1 day (20–40 mice per point). Curves were fitted by eye.

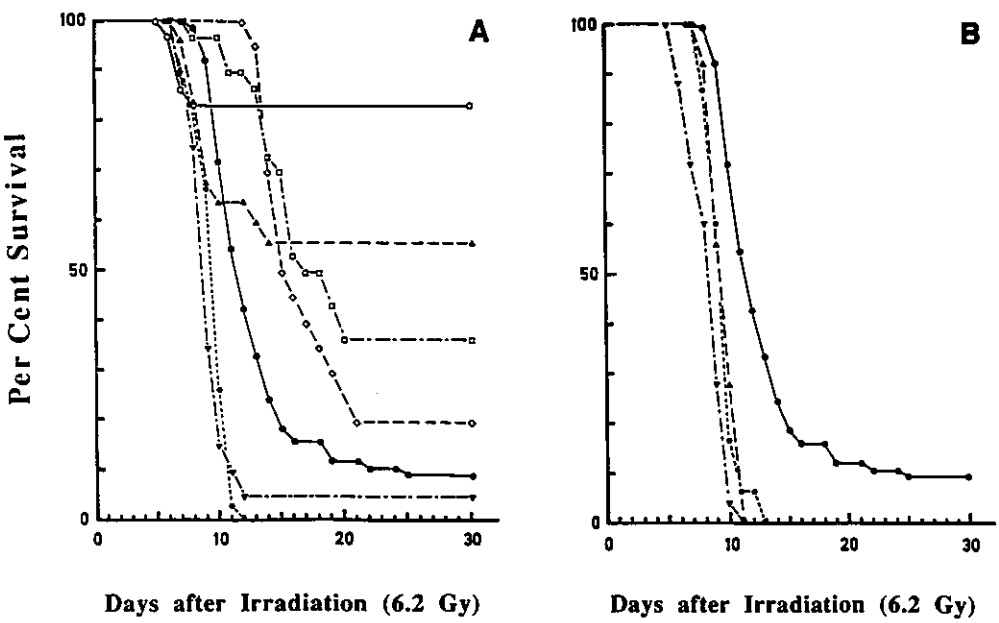


Fig. 2. Daily changes of per cent survival in mice during the 30 days after X-irradiation with 6.2 Gy. Panel A shows the changes of survival in mice with 5-FU treatment before irradiation. —●—, untreated controls (no 5-FU) (n=75 mice); --◇--, mice with 5-FU treatment at 15 days before irradiation (n=20 mice); -·□-·, 5-FU at 12 days (n=30 mice); —○—, 5-FU at 5 days (n=30 mice); --△--, 5-FU at 3 days (n=25 mice); -·▽-·, 5-FU at 1 day (n=40 mice); ····●····, 5-FU at 2 h (n=30 mice). Panel B shows the changes of survival in mice with 5-FU treatment after irradiation. —●—, untreated controls (the same data as those on panel A); ····●····, mice with 5-FU treatment at 2 h after irradiation (n=30 mice); -·▽-·, 5-FU at 1 day (n=25 mice); --△--, 5-FU at 3 days (n=25 mice).

RESULTS

Modification of radiation lethality by 5-FU Figure 1 shows the radiation-dose response curves for the 30-day survival of mice treated with 5-FU at various times (1, 3, 5, 12 and 15 days) before the irradiation with graded doses (4.8 to 7.6 Gy) of X-rays. The pretreatment with 5-FU at 5 days before irradiation was the most effective in lessening the lethality to irradiated mice, having a radioprotective effect. The X-ray dose corresponding to LD_{50} (30) was estimated as 7.3 Gy and 5.9 Gy for the group treated with 5-FU at 5 days before irradiation and the group given irradiation alone (untreated controls), respectively. The dose reduction factor (DRF) was obtained as 1.24. But, the treatment with 5-FU at 1 day before irradiation conversely decreased the survival compared to the untreated controls, having a radiosensitizing effect.

Figure 2 represents the quantitative changes of survival in mice during the 30 days after X-irradiation with 6.2 Gy. Mice were treated with 5-FU at various times before or after irradiation. Most of the untreated mice died from day 9 to 16 after irradiation, with average survival on day 30 at 9.3%. By contrast, the maximal rate of survival after 30 days was exhibited by mice treated with 5-FU at 5 days before irradiation, which had an 83.3% survival rate. 5-FU treatment at 3 days or 12 days before irradiation had reduced protective effects, with survival being 56.0% or 36.7% on day 30. However, the survival time of the animals that died in groups treated with 5-FU was shorter than that in the untreated controls between 5 and 10 days after irradiation. All of the mice with post-irradiation treatment died during the 5 to 13 days after irradiation (Fig. 2, B).

Radiosensitivity of endogenous CFU-S in mice treated with 5-FU before irradiation The response of endogenous CFU-S was examined as a function of radiation dose in mice with 5-FU treatment at 5 days, 3 days or 1 day before X-irradiation in the dose range from 4.8–6.7 Gy. The dose-effect survival curves are shown in Fig. 3. 5-FU treatment at 5 days before irradiation remarkably increased the number of endogenous CFU-S, but this effect was not seen in the group with 5-FU at 1 day. With pretreatment 3 days before irradiation, there was an increase of colony numbers only in the low dose range. When the radiation dose required to obtain 10 colonies

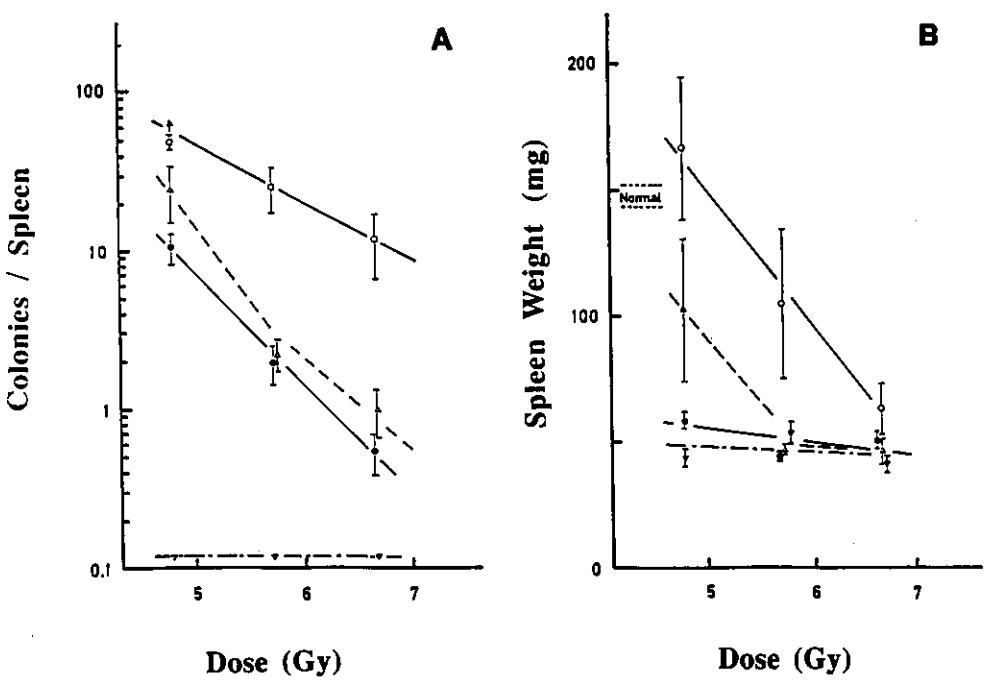


Fig. 3. Radiation-dose response curves for endogenous CFU-S (A) and spleen weight (B) in mice treated with 5-FU before X-irradiation. The number of colonies per spleen and spleen weight were plotted against radiation dose. —●—, untreated controls (no 5-FU); —○—, the group treated with 5-FU at 5 days before irradiation; --△--, the group treated with 5-FU at 3 days; --▽--, the group treated with 5-FU at 1 day. Each point represents the mean ± SE of 10–20 mice. Dotted lines (Normal) on panel B represent the mean ± SE of spleen weight in unirradiated normal mice (n=20 mice, 148.1±4.5).

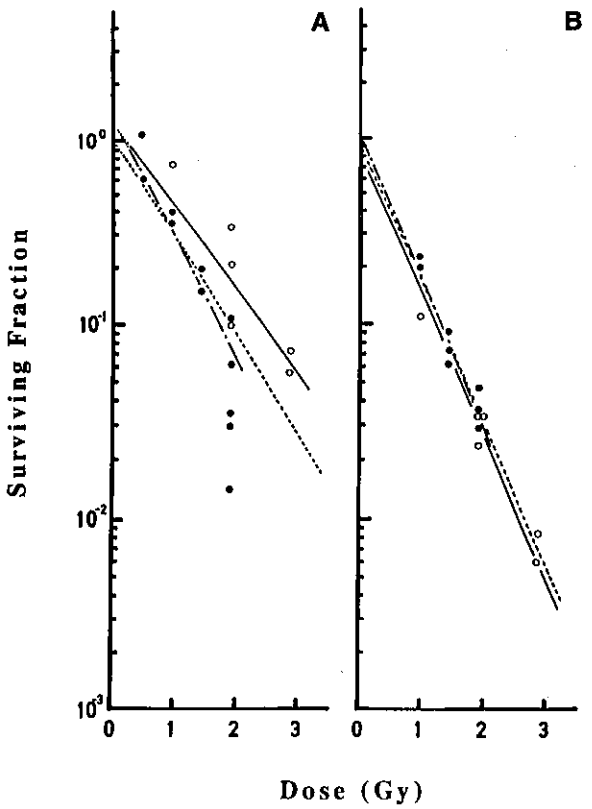


Fig. 4. Radiation-dose response curves for exogenous CFU-S in the femur (A) and spleen (B) of donor mice treated with 5-FU before X-irradiation. Surviving fractions of CFU-S were plotted against radiation dose. Dotted line, untreated controls (no 5-FU); open circles, the group treated with 5-FU at 5 days before irradiation; closed circles, 5-FU at 3 days. Different points denote the surviving fractions of CFU-S from separate experiments.

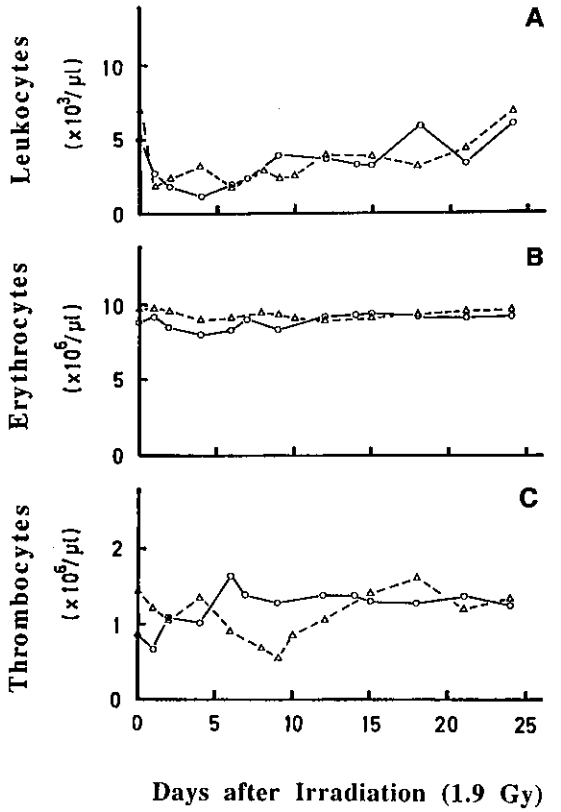


Fig. 5. Changes of leukocytes (A), erythrocytes (B) and thrombocytes (C) in the circulating blood after X-irradiation (1.9 Gy) in mice treated with 5-FU at 5 days before irradiation (circles) and in mice given irradiation alone (triangles). Each point represents the mean of 5–10 mice for the 5-FU-pretreated group and 6–9 mice for the irradiation alone group.

per spleen is compared between the group treated with 5-FU at 5 days before irradiation and the untreated controls, the DRF is estimated to be 1.4. This was similar to the radiation-dose relation for spleen weight (Fig. 3, B).

Radiosensitivity of exogenous CFU-S in mice treated with 5-FU before irradiation Figure 4 shows the radiation-dose response curves for exogenous CFU-S in the femur and spleen of donor mice treated with 5-FU at 5 days and 3 days before X-irradiation. The survival of CFU-S from donor mice was measured at 2 h after graded test exposures (0.5–2.9 Gy). The parameters of radiosensitivity of mice treated with 5-FU at 5 days before irradiation were larger than those of untreated controls, the D_0 and n values being 0.97 Gy and 1.3, and 0.84 Gy and 1.0, respectively. On the other hand, the survival curves of splenic CFU-S were not altered by 5-FU treatment (Fig. 4, B). The radiosensitivity of splenic CFU-S was almost the same for both the 5-FU treated groups and the untreated control groups (D_0=0.56–0.60 Gy, n=0.8–1.0). These data indicate that a small number of radioresistant clonogenic cells can be reserved in femoral CFU-S populations surviving for 5 days after 5-FU treatment.

Modification of radiation-induced changes in blood cell counts by 5-FU Figure 5 shows the patterns of change of leukocytes, erythrocytes and thrombocytes in the circulating blood after X-irradiation (with a dose of 1.9 Gy) in mice treated with 5-FU at 5 days before irradiation and in mice given irradiation alone. The post-irradiation patterns of leukocyte and erythrocyte variation did not show any significant change with pretreatment, although there were slight changes in leukocyte counts with 5-FU treatment on days 9 and 18. By contrast, the change of thrombocyte counts was greatly modified by

the pretreatment with 5-FU. That is, treatment with 5-FU was effective in lessening the radiation-induced depression of thrombocyte numbers during 6 to 14 days after irradiation, when thrombocytes in untreated mice (irradiation alone) showed lower levels than in normal controls. These results indicate that the recovery from radiation-induced injury to thrombocytes can be accelerated by 5-FU pretreatment. Also, the previous study has indicated that the administration of 5-FU alone into normal mice results in mild thrombocytopenia, followed by a marked rebound in thrombocytosis, and by increase in the number of megakaryocyte colony-forming cells.[12, 13] There are no discrepancies between the present results and those of the previous study.

DISCUSSION

Our data indicate that: (1) the survival rate of irradiated mice increased most on day 30, (2) the number of endogenous CFU-S increased remarkably, (3) exogenous CFU-S in femur, but not in spleen, became radioresistant, and (4) the post-irradiation recovery of thrombocytes in the circulating blood was accelerated when 5-FU was administered to mice at 5 days before X-irradiation. Therefore, the appearance of radioresistant CFU-S, the increase of repopulating ability in CFU-S populations and lastly a sufficient supply of circulating thrombocytes in irradiated mice might result in the enhancement of survival in mice pretreated with 5-FU at an optimal time before irradiation.

Such enhancements of survival have been observed in animals treated with other cytotoxic agents such as vinblastine, vincristine,[14] cytosine arabinoside[15] and methotrexate.[15, 16] In those cases, the timing suitable for a maximal protective effect due to pretreatment with the cytotoxic agents was 1 to 3 days before irradiation. It has been demonstrated that the increased survival in animals treated with such cytotoxic agents results from the accelerated recovery of hematopoietic stem cells.[15, 17] The present report indicates that the maximal radioprotection of irradiated mice occurred when they had been treated with 5-FU at 5 days before irradiation. This time difference for optimum efficacy among the above agents could be due to different action mechanisms. It has been reported by von der Maase[16] that 5-FU dramatically decreases the survival (with an LD_{50} (28) of 0.75 Gy) when administered to mice at 15 min before irradiation. But, the change of radiation lethality was time-dependent, the lethality being lower in mice given 5-FU 1–3 days before than 1–3 days after irradiation. Accordingly, the present results are essentially consistent with that report. Our previous results showed that the number of CFU-S after 5-FU treatment alone reached the minimal and the maximal values (over untreated control values) on day 3 and day 12, respectively (not shown). When 5-FU was administered to mice at 3 days or 12 days before irradiation, the survival rates increased as well, but with moderate radioprotective effects. In contrast, we could not demonstrate greater radioresistance for mice treated with 5-FU at 12 days before irradiation, in spite of having abundant CFU-S at the time of irradiation. Thus, it is conceivable that the period of 5 days or so after 5-FU treatment may be a specific period when primitive stem cells provide radiation response. These results suggest that a small number of radioresistant cells exist in the hematopoietic stem cell population and they have a high repopulating ability. A recent study showed that most primitive hematopoietic stem cells are stimulated to proliferate rapidly 3 to 5 days after 5-FU treatment alone in normal mice.[18] It is necessary to analyze in more detail the nature of hematopoietic stem cells during the period after 5-FU treatment.

It has been well documented that radiation-induced thrombocytopenia is one of the main causes in the development of hematopoietic death.[19–22] Our results show that optimal pretreatment with 5-FU lessens the post-irradiation depression of circulating thrombocytes. It is known that vincristine,[23–25] as well as 5-FU,[6, 12, 13] stimulates megakaryocytopoiesis and results in the enhancement of thrombocyte production in normal animals. It was also reported that cytosine arabinoside enhanced the regeneration of thrombocytes in irradiated mice when administered at 2 days before irradiation, compared with that in mice given irradiation alone.[15] Consequently, it is possible that the alleviation of radiation-induced thrombocytopenia in mice pretreated with 5-FU could result in the reduction of radiation lethality of irradiated mice.

Radiation-induced deaths were accelerated in mice treated with 5-FU after irradiation, even if the mice were given a dose which caused hematopoietic death (Fig. 2, B). Chadwick and Rogers[26] reported that the disposition of 5-FU and its metabolites was observed not only in bone marrow and spleen, but also in small intestine and liver in 5-FU-treated mice. Therefore, the accelerated mortality in mice treated with 5-FU after irradiation might be associated with the radiation injury of life-maintaining organs other than hematopoietic organs, although the possibility of synergetic effects of radiation and 5-FU can not be excluded. Studies of this question are in progress.

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