Low-Dose Whole-Body Irradiation Induced Radioadaptive Response in C57BL/6 Mice

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Radioadaptive survival responses after relatively low doses of radiation were investigated in C57BL/6 mice. The 8-week-old mice received whole-body mid-lethal challenging irradiation (5.9 Gy) at various intervals after conditioning whole-body irradiation with 50–400 mGy. Thereafter, survival of the mice was observed for 30 days. The mice receiving 400 mGy at 6 h before the challenging dose had a lower survival rate than the control group, but it was not observed when the conditioning 400-mGy irradiation was given 24 h beforehand. The conditioning doses of 100 and 200 mGy did not influence the survival of mice after the challenging dose. The mice receiving 50 mGy at 1 day, 3 days or 1 week before the challenging dose had a higher survival rate than the control, although this adaptive response was not observed when 50 mGy was given 6 h, 12 h, 3.5 weeks, or 5 weeks beforehand. When 50 mGy was given 2 weeks before the challenging dose, the adaptive response was observed in an experiment in which the mice were caged in our laboratory at the age of 5 weeks, whereas it was not observed in another experiment in which the mice were caged at 3 weeks. This study confirmed the presence of radioadaptive survival responses at the dose of 50 mGy given relatively shortly before the challenging dose.

INTRODUCTION

With the development of diagnostic imaging and interventional radiology, there is an increasing interest in the effects of low-dose (≤200 mGy) radiation. Until recently, the risks of detrimental effects from exposure to low-dose radiation have been estimated by extrapolation from data obtained at high-dose radiation, using the linear no-threshold (LNT) model. However, much criticism has grown against the LNT theory since the introduction of “radiation hormesis” hypothesis indicating various beneficial effects of low-dose radiation on living organisms.1–4) Indeed, considerable amounts of evidence have accumulated, suggesting that living organisms, including humans, might respond differently to low-dose radiation than they do to high-dose radiation.

Radioadaptive response as defined by the induction of radioresistance to subsequent higher doses of radiation by pretreatment with low radiation doses is one of beneficial effects of low-dose radiation. It has been reported by many investigators, since Olivieri et al.5) first documented the phenomenon in 1984. Azzam et al.6) reported that a low-dose-rate pre-exposure to ionizing radiation induced an adaptive response in C3H 10T1/2 mouse embryo cells and that this response enhanced DNA double-strand break repair when cells were subsequently exposed to a second radiation dose. Adaptive responses have been observed in various organisms including bacteria, lymphocytes, cultured cells, and mice, but most studies have been carried out in vitro.7–12) In addition, the response is not necessarily recognized in every situation, and it is not clear which factors contribute to induction of adaptive response.13–15)

In vivo, radioadaptive response with respect to survival rate after high-dose irradiation has been reported by Yonezawa et al.16–19) They reported two types of radioadaptive survival response against subsequent lethal-dose radiation in mice. One was induced by a conditioning dose of 50 mGy and radioadaptive response was observed 2 months later, and the other was induced by 500 mGy given 2 weeks before the second challenging dose. However, such in vivo radioadaptive responses have been scarcely reported by other investigators. Moreover, it has not been clear whether it develops at shorter intervals (e.g., within 24 h). This should be an important issue to be investigated, because in vitro adaptive responses have been observed shortly (within 12 h) after conditioning radiation.6,7,10) In this study, therefore, we attempted to clarify whether or not such a radioprotective
Effect was observed in C57BL/6 mice at shorter intervals after conditioning whole-body irradiation using relatively low doses of 50–100 mGy that are frequently used in diagnostic radiology.

MATERIALS AND METHODS

Animals

C57BL/6Cr Slc mice at the age of 3–7 weeks purchased from Nihon SLC Co. Ltd. (Hamamatsu, Japan) were used. They were maintained under specific pathogen-free conditions at 24°C ± 2°C and 60% ± 10% relative humidity, and were provided with nutritional chow (CRF-1, Charles River Laboratories Japan Inc., Yokohama, Japan) and reverse osmosis (RO) water ad libitum. The RO water rejects approximately 95% to 99% of all ionic materials including bacteria, viruses and pyrogens. Before giving conditioning irradiation, usually 8 mice were housed in a sterile polypropylene cage containing a sterile paddy as bedding. After the conditioning dose, 4 mice were caged together. We intended to use male mice throughout the study. However, since male mice tended to fight and injure each other, especially before giving the challenging dose, we had to use female mice in the experiments in which the interval between caging and conditioning radiation was 2 weeks or longer (fourth and fifth experiments). The use of experimental animals of this study has been reviewed and approved by an ethics committee for animal experiments at Nagoya City University.

Irradiation

In all experiments, the mice were whole-body irradiated in a plastic chamber with an X-ray machine CAX-210 (Chubu Medical Co. Ltd., Yokkaichi, Japan; 210 kV, 0.5 or 10 mA, 2-mm Al filter) without physical restraint or anesthesia. This machine has been used in our previous biological studies and radiation procedure has been described in more details. A universal dosimeter, RAMTEC 1000 with an N300001 chamber (Toyo Medic Co. Ltd., Tokyo, Japan), was used for the dosimetry. The dose rates were 40 mGy/min for low-dose conditioning irradiation (≤ 400 mGy) and 1 Gy/min for challenging or high-dose irradiation. All challenging or high-dose irradiation was given to 8-week-old mice.

Survival studies

First, a preliminary experiment was performed to determine the 50% lethal dose in C57BL/6 mice using 35 mice. Seven mice each were irradiated to the whole body at 5, 5.5, 6, 6.5, and 7 Gy, and their survival was observed for 30 days. The radioadaptive survival response was examined by comparing survival curves of mice exposed to conditioning preirradiation or sham-radiation followed by mid-lethal challenging irradiation. The conditioning dose, the challenging dose, and the interval between the two courses of radiation were as follows. The mice received a single conditioning dose of 50, 100, 200, or 400 mGy. Control groups were sham-irradiated. At 6 or 24 h intervals, the mice were exposed to a mid-lethal challenging dose (5.9 Gy). In the following experiments, the mice received a single dose of 50 mGy at 12 h, 24 h, 3 days, 1 week, 2 weeks, 3.5 weeks, or 5 weeks before the challenging dose. Forty or 50 mice were used for each group.

Survival curves of mice were generated by the Kaplan-Meier method and differences between pairs of survival curves were examined by the logrank test using a computer program PRISM version 4.0c (GraphPad Software, Inc., San Diego, CA, USA).

RESULTS

The 50% lethal dose in the C57BL/6 mice was estimated to be 5.9 Gy (95% confidence interval: 5.6–6.2 Gy). Subsequently, all challenging irradiation was therefore given at 5.9 Gy. Figure 1 shows survival curves of mice following whole-body irradiation at 5.9 Gy with sham irradiation given 6 or 24 h beforehand. There was no difference in survival of mice between the two groups. Based on this result, sham irradiation was given 6 h before challenging irradiation in the subsequent 2 experiments.

Figure 2 shows survival of mice following 5.9-Gy irradiation with or without a relatively high conditioning dose (200 or 400 mGy) delivered 6 or 24 h beforehand. The mice receiving 400 mGy 6 h before the challenging dose had
lower survival rates than the control (sham-irradiated) group ($p = 0.0032$). The group receiving 200 mGy 6 h beforehand tended to have lower survival rates but the difference was not significant. The other two groups had survival rates similar to those for the sham-irradiated group.

Figure 3 shows survival of mice following 5.9-Gy irradiation with or without a relatively low conditioning dose (50 or 100 mGy) delivered 6 or 24 h beforehand. The group receiving 50 mGy 24 h before the challenging dose had significantly higher survival rates than the control group ($p = 0.021$). Although survival curves for the other preirradiated

![Fig. 2. Survival curves for C57BL/6 male mice after conditioning irradiation at 0, 200 or 400 mGy and challenging irradiation at 5.9 Gy given 6 or 24 h later. Each group consisted of 40 mice. Mice were caged at the age of 7 weeks and the challenging dose was given at 8 weeks.](image)

![Fig. 3. Survival curves for C57BL/6 male mice after conditioning irradiation at 0, 50 or 100 mGy and challenging irradiation at 5.9 Gy given 6 or 24 h later. Each group consisted of 50 mice. Mice were caged at the age of 7 weeks and the challenging dose was given at 8 weeks.](image)

![Fig. 4. Survival curves for C57BL/6 female mice after conditioning irradiation at 50 mGy and challenging irradiation at 5.9 Gy given 12 h, 24 h, 3 days, 1 week or 2 weeks later. Each group consisted of 40 mice. Mice were caged at the age of 5 weeks and the challenging dose was given at 8 weeks.](image)

![Fig. 5. Survival curves for C57BL/6 female mice after conditioning irradiation at 50 mGy and challenging irradiation at 5.9 Gy given 2, 3.5, or 5 weeks later. Each group consisted of 40 mice. Mice were caged at the age of 3 weeks and the challenging dose was given at 8 weeks.](image)
groups lay above the curve for the control group, the differences were not significant.

In the next experiments, influences of intervals between the two radiation courses were investigated using a 50-mGy conditioning dose. As shown in Fig. 4, the mice receiving 50 mGy 1, 3, 7 or 14 days before the challenging dose had significantly higher survival rates than the control sham-irradiated group, while the mice receiving the dose 1 h before the challenging dose had survival rates similar to those of the control group.

To further investigate adaptive responses at longer intervals between the two radiation doses, the mice were irradiated with a priming dose of 50 mGy 2, 3.5, or 5 weeks before the challenging dose. With these intervals, however, no radioadaptive survival responses were observed (Fig. 5).

**DISCUSSION**

Radioadaptive survival responses in mice have been reported by Yonezawa et al.16–19 The present study, however, differs from their works in that challenging irradiation was given to the mice at the age of 8 weeks throughout the experiments; Yonezawa et al.16–19 gave conditioning irradiation at 6 weeks of age in experiments using ICR mice16,17 and at 8 weeks for C57BL/6 mice.18 and challenging irradiation was given at various ages. Radiosensitivity of mice has been reported to vary with age, and some mouse strains are reported to be most radiosensitive at puberty.24,25 So, mice ages at challenging irradiation may have a greater influence, if any, upon survival rates than ages at conditioning irradiation. Therefore, we considered that giving the challenging dose at the same age might be more important than giving the conditioning dose at the same age. In addition, since Yonezawa et al.17 reported radioadaptive responses at relatively longer intervals (2–2.5 months) after conditioning irradiation at 50–100 mGy but did not prove the phenomenon at shorter intervals in ICR mice, we investigated the radioadaptive response at shorter intervals in more details.

In this study, we observed radioadaptive responses in C57BL/6 mice following a radiation dose of 50 mGy. At higher doses, adaptive response was not observed when conditioning radiation was performed 6 or 24 h before challenging radiation. Moreover, the mice receiving 400 mGy at 6 h before the challenging dose had a lower survival rate than the control groups; this should be simply due to the higher total dose (6.3 Gy vs 5.9 Gy). This effect was not observed when 400 mGy was given 24 h beforehand, the reason may be the recovery from sublethal damage of hematopoietic cells. If so, recovery from sublethal damage might not have completed within 6 h after the conditioning irradiation. Tiku and Kale26 observed an adaptive response in Swiss albino mice after a conditioning dose of 250 mGy given 6 or 24 h before challenging irradiation, but this was not observed when 500 mGy was given at the same timings. Their results are somewhat in contrast to ours; this discrepancy may be due to the mouse strain used, but the number of mice used in their study was smaller (18 to 31 per group) than that used in the present study (40 or 50 per group).

In the third experiment in the present study (Fig. 3), apparent radioadaptive response was observed when 50 mGy was given 24 h before the challenging dose. It was confirmed in the next experiment in which 50 mGy was given at 1, 3, 7 or 14 days before the challenging dose (Fig. 4). In the subsequent fifth experiment (Fig. 5), the adaptive response was not observed when 50 mGy was given at 2, 3.5 or 5 weeks before 5.9 Gy. Therefore, there was a discrepancy between the results of the two experiments with respect to the effect of 50 mGy given 2 weeks beforehand. Since all the mice were given the challenging dose at the age of 8 weeks, the mice were caged in our laboratory at the age of 5 weeks in the fourth experiment and at 3 weeks in the fifth experiment. Such a subtle difference in breeding conditions may influence development of radioadaptive response. After a conditioning dose of 50 mGy, Yonezawa et al.16–19 observed radioadaptive responses at 2 to 2.5 months but not at 1 day to 1.5 months in ICR mice. They observed radioadaptive response in C57BL/6 mice at the same conditioning dose and interval.19 In the present study, we demonstrated the presence of adaptive response at 1 to 14 days after a conditioning dose of 50 mGy, but we could not investigate the intervals longer than 5 weeks because our study design was to give the challenging dose at the age of 8 weeks throughout the study. Since the mice strain, age at conditioning and challenging radiation, sex, and breeding conditions may influence development of radioadaptive responses, it may not be surprising that we observed adaptive responses at intervals shorter than those reported by Yonezawa et al.16–19 Also, Yonezawa et al.17,18 reported a radioadaptive response following 300–500 mGy given 9 to 17 days before the challenging dose. Since the present study mainly attempted to clarify radioadaptive responses relatively shortly after conditioning radiation with relatively low doses of radiation, we did not attempt to reproduce the results of Yonezawa et al.

In our study, male mice were used in the first 3 experiments, whereas female mice were used in the following 2 experiments, because male mice tended to fight and injure each other when caging periods became longer. To use mice of the same sex is desirable, but fortunately, no difference in survival after lethal irradiation due to sex has been reported for C57BL/6 mice.27 Also, Yonezawa et al.17 observed radioadaptive response in both genders. Similarly in our study, adaptive response after 50 mGy given 24 h before the challenging dose was observed in both males and females (third and fourth experiments). Survival rates for control mice somewhat differed with experiments between 35% and 55% at 30 days, and the difference in survival rates was significant between the fourth and fifth experiments (p = 0.044). However, since the radiation conditions were identi-
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cical in all experiments, comparison of survival rates using mice of the same sex in each experiment was considered meaningful.

According to Yonezawa et al., adaptive responses seen 9 to 17 days after conditioning radiation at 500 mGy is considered to be due mainly to recovery and increase of hematopoietic cells. In the present study, adaptive response was observed 1–14 days after 50 mGy so that other mechanisms should also be considered. Various mechanisms have been considered to be involved in development of adaptive responses, including induction of antioxidative and DNA repair enzymes, HSP72 and radioprotective substances such as glutathione, and enhancement of immune responses. Some of these mechanisms may be activated shortly after low-dose radiation. We are now investigating glutathione levels in serum and tissues after whole-body radiation in mice. Radioadaptive response has been reported to be related to the p53 status; it is generally observed in living organisms with wild-type p53. Takahashi observed that accumulation of p53 which occurs after high-dose-rate radiation (1 Gy/min, 5 Gy) was strongly suppressed by priming low-dose-rate radiation (0.001 Gy/min, 1.5 Gy). Also, p53-dependent apoptosis after exposure to high-dose-rate radiation was found to be suppressed by priming low-dose-rate radiation in cultured cells and in the spleens of mice. Ohnishi et al. observed increases in p53 and WAP1 protein levels within 6 h after low-dose-rate radiation but they returned to pretreatment or even lower levels at 25 h or later. Matsubara et al. found striking increases in the spleen plaque-forming cell count in ICR mice 1 day after stress-inducing pretreatment with manganese chloride or OK-432 injection or skin excision. Such processes may also be related to the early development of adaptive responses. Anyhow, mechanisms of radioadaptive responses should be further clarified in future studies.

In summary, our study revealed a radioadaptive response in vivo at 1 day to 2 weeks after a low dose of 50 mGy of whole-body radiation. Radioadaptive survival responses at this dose level and these intervals after a conditioning dose have not been reported by other investigators. Further studies on the effect of low-dose radiation seem to be strongly recommended in order to extinguish unreasonable fear against low-dose ionizing radiation in the field of medicine.

REFERENCES


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