Fractionated Irradiation Augments Inter-Strain Variation of Skin Reactions among Three Strains of Mice

Toshie OHTA, Mayumi IWAKAWA*, Chisa OOHIRA, Shuhei NODA, Yang MINFU, Miyako GOTO, Hiroko TANAKA, Yoshinobu HARADA, and Takashi IMAI

Radiosensitivity/Strain Difference/Fractionation/Skin Reaction.

The multifraction regimens commonly used in conventional clinical radiotherapy are largely based on radiobiological experiments. However, no experimental reports on skin reactions focusing on inter-strain differences have displayed clinical relevance to the fractionated dose schedule. In this study, mice of inbred strains A/J, C57BL/6J, and C3H/HeMs were used to reveal inter-strain difference after multifractionated irradiation. Irradiation was performed daily at graded doses of 30–60 Gy total doses, with 10 fractions of 3–6 Gy. Acute skin reactions following irradiation were scored for 50 days after irradiation. Dividing a dose into a number of fractions obviously spared skin damage in the three strains of mice. No mouse exhibited a skin damage score more than 1.5, while single dose irradiation resulted in skin damage scores up to 3. The three different strains, however, showed varying susceptibility to fractionated irradiation within the range under 1.5. C3H/HeMs did not display any skin reaction after irradiation with 40 Gy total dose, while C57BL/6J and A/J demonstrated various skin reactions. Different latent periods of damage were also observed among the strains after irradiation at each dose.

Our data suggest that genetic factors cause obvious variations in severity of damage and latent period after fractionated irradiation.

INTRODUCTION

Published reports about skin reactions to radiotherapy, particularly among breast-cancer patients, also suggest the existence of interindividual differences in normal tissue response, and genetic factors are thought to be involved.\(^1\)\(^-\)\(^4\) The literature indicates that mouse models offer a possible experimental pathway for understanding inter-individual differences in radiosusceptibility.\(^5\)\(^-\)\(^10\)

Clinically, multifraction regimens commonly used in conventional radiotherapy are largely based on radiobiological experiments. Dividing doses into smaller fractions spares normal tissues by allowing repair of sublethal damage between dose fractions and repopulation of cells if the overall time is sufficiently long. However, no experimental reports on skin reaction focusing on inter-strain differences have displayed clinical relevance to the fractionated dose schedule. We recently reported inter-strain differences in single dose radiation susceptibility among 5 murine strains (A/J, C3H/HeMs, C57BL/6J, C. B.17/ICr-scid and C3H-scid) using the functional endpoint of skin reaction as indicating acute response.\(^11\) This variation in macroscopic and histopathological changes in skin reaction observed between murine strains during the progression and resolution of irradiation damage suggests that inter-strain variation of radiosusceptibility may be caused by genetic factors involved in all processes of injury, repair and remodeling of tissue.

The present study examined whether the same inter-strain differences detectable in skin reactions after single dose irradiation would be observed in skin reactions after fractionated irradiation.

MATERIALS AND METHODS

Mice

Female mice of inbred strains A/J, C57BL/6J, and C3H/HeMs were used throughout the experiments. Mice were obtained and maintained from specific-pathogen-free mice colonies at the National Institute of Radiological Sciences. Mice were 12-weeks-old at time of irradiation. A maximum of 6 mice were kept in each cage. To examine macroscopic
skin reactions, 6 mice from each strain received one of the graded total doses of fractionated irradiation. To examine skin histopathology, 3 mice from each strain were irradiated under a fractionation schedule and sacrificed by spinal dislocation at 10, 20 and 30 days after irradiation. A total of 108 mice were used in this study. Methods were reviewed and approved by the NIRS Institutional Animal Care and Use Committee, under protocol number 13–1073.

Irradiation
Skin of the right hind leg was chemically depilated 5 days before irradiation. Local irradiation was performed using a beam of Cs-137 gamma-rays at an FSD of 21 cm with a dose rate of 1.4 Gy/min. A toroidal magnetic field with a 30-mm rim was used to collimate the vertical beam. Mice were anesthetized with pentobarbital at a dose of 50 mg/kg body weight before irradiation. Adhesive tape was used to immobilize the mice on a Lucite plate and position their right hind legs. Irradiation was performed daily at graded fractionated doses of 30–60 Gy over 12 days, in 10 fractions of 3–6 Gy. Six mice of each strain were left unirradiated to serve as controls.

Skin Reactions
Acute skin reactions following graded fraction doses of gamma-rays were scored every other day for 50 days after irradiation, using the arbitrary scale shown in Table 1. The scoring system comprised 10 degrees, from 0.5 to 3.5. The peak skin reaction score at each dose group was plotted for each strain to obtain a regression line.

Histopathology
Irradiated skin fragments of 2 cm² were examined for histopathology, 3 mice from each strain were irradiated under a fractionation schedule and sacrificed by spinal dislocation at 10, 20 and 30 days after irradiation. A total of 108 mice were used in this study. Methods were reviewed and approved by the NIRS Institutional Animal Care and Use Committee, under protocol number 13–1073.

Skin Reactions
Acute skin reactions following graded fraction doses of gamma-rays were scored every other day for 50 days after irradiation, using the arbitrary scale shown in Table 1. The scoring system comprised 10 degrees, from 0.5 to 3.5. The peak skin reaction score at each dose group was plotted for each strain to obtain a regression line.

Histopathology
Irradiated skin fragments of 2 cm² were examined for histopathology, 3 mice from each strain were irradiated under a fractionation schedule and sacrificed by spinal dislocation at 10, 20 and 30 days after irradiation. A total of 108 mice were used in this study. Methods were reviewed and approved by the NIRS Institutional Animal Care and Use Committee, under protocol number 13–1073.
topathological changes. Skin fragments were fixed in 10% neutralized formalin and embedded in paraffin. Sections were cut at a width of 3 micrometer and stained with hematoxylin and eosin (H&E).

RESULTS

The three different strains showed varying susceptibility to fractionated irradiation (Fig. 1 A–C). Comparison of time course for skin reactions after fractionated irradiation with a total dose of 40 Gy yielded the following order of radiosensitivities: C57BL/6J, A/J, and C3H/HeMs, from most to least sensitive. Different latent periods for damage were also observed among the three strains after irradiation at each dose. A/J mice were the slowest responders for presenting skin reaction after irradiation at a total dose of 60 Gy in 10 fractions. C3H/HeMs did not display any skin reaction after irradiation at a total dose of 40 Gy, while C57BL/6J or A/J demonstrated various skin reactions (Fig. 1).

Dose-response analysis using the peak skin reaction score in each total dose group resulted in a nearly identical regression line among the three strains (Fig. 2). Mean slope of the linear equation for fractionated irradiation was 0.04 (range, 0.035–0.042). On lower doses of 30–40 Gy total dose, C3H/HeMs mice were obviously more resistant than the other 2 strains.

Histological examination revealed disrupted epidermis and reactive thickness of dermis in the three strains of mice after irradiation. A skin flap within irradiated field of hind leg was taken at 10, 20, or 40 days after commitment of irradiation in each strain group with each dose group for histological examination. Figure 3A demonstrated ulcer formation and disrupted epidermis with layers of dermis in A/J mice at 10 days after commitment of irradiation with 10 fractions of 6 Gy. More layers of dermis were observed in C3H/HeMs mice with lymphocyte infiltration in connective tissue under dermis (Fig. 3B). Severely damaged epidermis without ulcer was observed in C57/BL/6J mice with modest infiltration of lymphocytes in connective tissue (Fig. 3C). C3H/HeMs mice appeared the most resistant through scoring system for skin surface, but microscopic examination revealed that C3H/HeMs mice experienced the most dynamic rea-

---

**Fig. 2.** Peak skin-reaction score at each dose for the following 3 strains: (solid circle) A/J mice; (open circle) C3H/HeMs mice and (solid diamond) C57BL/6J mice.

**Fig. 3.** H&E-stained sections of (A) A/J, (B) C3H/HeMs and (C) C57BL/6J mouse skin 10 or 11 days after commitment of irradiation.
tion in deeper layers, with marked influx of inflammatory cells.

**DISCUSSION**

Most animal models involve irradiation of the whole animal with single, or large doses of radiation. This complicates the application of such data to human beings, where treatment is usually administered in multiple smaller doses over an extended period. During clinical cancer treatment, the therapeutic efficacy of radiation is increased by delivering 20–30 fractions of 2–3 Gy each, spread over 5–6 weeks. Although the therapeutic gains are immense, normal skin invariably suffers from the cytotoxic effects of radiation. The present study was designed to investigate the genetic contribution to irradiation-induced skin reactions following fractionated irradiation and has clinical relevance, as a fractionated dose schedule similar to a clinical radiotherapeutic regimen has been followed to evaluate the adverse effects of radiation with inter-strain differences.

Several papers have examined animal models using fractionated irradiation. Some studies have revealed a fractionated schedule to identify repair capacity between split doses. Dividing a dose into a number of fractions obviously spared skin damage in this research. No mouse exhibited a skin damage score more than 1.5, while single dose irradiation resulted in skin damage scores up to 3. This benefit of fractionation did not diminish inter-strain differences within the range of 30–60 Gy in the present study. Our data indicate that genetic factors cause marked variations in degree of damage and latent period, even with an identical regression equation for dose-response analysis among the three strains.

The fact that inter-strain difference in this study affected only the part of the dose-response curve at lower doses is noteworthy. Inter-strain variations following fractionated irradiation did not significantly shift the dose-response curve of radiation-induced skin reactions among the three strains. In general, drawing isoeffective curves for skin reactions such as necrosis, moist desquamation, dry desquamation, or erythema to be parallel slopes might prove useful. However, drawing isoeffective curves for each skin reaction was difficult using our data, as only skin reaction scores of 0.5 or 1 were observed after fractionated irradiation at total doses of 50 or 60 Gy among all three strains.

In radiation biology, the mechanisms of bystander effect have been gaining attention. Fractionated irradiation stimulates the accumulation of bystander effect. Garcia-Barros et al. recently reported that tumor response to radiation is determined not only by tumor cell phenotype, but also by normal tissue sensitivity. Interindividual radiosensitivity of normal tissues might display some correlations with clinical tumor curability itself.

The present study investigated the impact of genetic differences on the effects of fractionated irradiation. Our data suggest that genetic factors cause variations in severity of damage and latent period after fractionated irradiation, even with an identical regression equation for dose-response analysis.

**CONCLUSION**

Inter-strain differences observed in skin reactions after fractionated irradiation were investigated. Female mice of inbred strains A/J, C57BL/6J, and C3H/HeMs were irradiated daily with graded fractionated doses of 30–60 Gy total dose for 12 days, with 10 fractions of 3–6 Gy. C3H/HeMs mice were noticeably more resistant than the other strains. Different latent periods of damage were observed between the three strains after irradiation at each dose.

**ACKNOWLEDGEMENTS**

We wish to express our deep thanks to Mr. Tatsuo Hayao and Mrs. Yuriko Ogawa for their animal-care services.

**REFERENCES**


Received on February 12, 2004
1st Revision on June 18, 2004
Accepted on August 9, 2004