

Hypersensitivity of LEC Strain Rats in Radiation-Induced Acute Bone-Marrow Death

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ABSTRACT. LEC strain rats, which have been known to develop hereditarily spontaneous fulminant hepatitis 4 to 5 months after birth, were highly sensitive to whole-body X ray-irradiation as compared to WKAH strain rats. Radiation-induced acute bone-marrow death occurred at doses higher than 2.0 Gy in LEC rats, and at doses higher than 7.4 Gy in WKAH rats, respectively. By probit analysis of survival data, it was shown that the LD_{50/30} value for LEC rats was 3.0 Gy which was significantly lower than that (7.8 Gy) of WKAH rats. Histopathological examinations of the bone marrows from both strains after irradiation at a dose of 4.0 Gy revealed that a number of hemopoietic cells were recovered in WKAH rats on day 8 after irradiation, but not in LEC rats. These results suggested the hypersensitivity of LEC rats to ionizing radiation in connection with acute bone-marrow death.—**KEY WORDS :** acute bone-marrow death, hypersensitivity, LD_{50/30}, LEC strain rat, radiation death.

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An inbred LEC strain has been established from a closed colony of Long-Evans rats at the Center for Experimental Plants and Animals, Hokkaido University [20]. Spontaneous fulminant hepatitis associated with severe jaundice occurs in about 80% of LEC rats between 4 to 5 months of age and most of the affected rats die within 2 weeks after the onset of jaundice [14, 20]. The clinical signs of such hepatitis in LEC rats resemble those of human fulminant hepatitis. Recently it has been reported that a dense copper element accumulation occurs in the liver of LEC rats, while their serum levels of copper and ceruloplasmin are rather reduced [12, 13]. The LEC rat therefore provides an animal model for human Wilson's disease [5].

We previously reported that LEC rats were more sensitive to radiation-induced acute intestinal death than other strains of rats [8]. The LD_{50/7} value in LEC rats was estimated to be 7.03 Gy which was significantly lower than that (12.99 Gy) of WKAH rats. Histopathological examinations of the small intestines from LEC rats on 2-day after irradiation at 8.5 Gy showed severe epithelial death together with edema, whereas little or no significant changes were noted in the intestinal epithelium of WKAH rats irradiated with the same dose. Preliminary results indicated that LEC rats also died earlier and had a higher mortality rate following X ray-irradiation at doses lower than 5 Gy, while WKAH rats did not die. Such lower doses could induce acute bone-marrow death [3]. For example, in mice which

died from bone-marrow death, higher radiation sensitivity of hemopoietic stem cells and the relationship of endogenous bacterial flora to radiation-induced death have been observed. Therefore, in the present study we examined the radiation-induced damage to the bone marrow in LEC rats induced by X ray-irradiation in order to investigate whether or not LEC rats are more radiosensitive and subject to acute bone-marrow death than other strains of rats. As Wistar strain rats are often used in the field of experimental radiation biology [4, 10, 23], we compared the radiosensitivity of LEC rats with that of inbred Wistar strain (WKAH) rats.

MATERIALS AND METHODS

Rats: Male LEC/Hkm (LEC) and WKAH/Hkm (WKAH) rats were maintained at the Institute for Animal Experimentation, Hokkaido University. They were housed in plastic cages on hardwood chip contact bedding, and allowed food and water *ad libitum*. Animal holding rooms were maintained at 22±3°C and 50±10% relative humidity with 17 cycle changes per hour of 50% conditioned fresh air and exposed to full spectrum light from 7:00 a.m. to 7:00 p.m. All the rats used in this study were 8 weeks old. Research was conducted according to the principles shown in the "Guide for the Care and Use of Laboratory Animals" prepared by Hokkaido University.

X ray-irradiation: Since circadian rhythmicity in

the radiosensitivity of animals to whole-body irradiation has been noted [9, 18], irradiation was always performed between 9:00 a.m. and 11:00 a.m. in the present study. Rats were irradiated at a dose rate of 0.69 Gy/min with a Toshiba KXC-18 X-ray generator operating at 160 kVp and 25 mA with a 0.5 mm Al + 0.5 mm Cu filter. After whole-body exposure to X-rays, the animals were inspected for survival conditions at least twice a day. A group of ten normal unirradiated rats served as the control.

Calculation of $LD_{50/30}$: The radiosensitivity of the irradiated animals was expressed in terms of $LD_{50/30}$, a dose with which 50% of irradiated individuals will die within 30 days. The calculation of $LD_{50/30}$ was done by the maximum likelihood method on the Probit-dose curve, and their confidential limits were evaluated by Fieller's formula as described [22].

Histopathology: The rats were anaesthetized with ether and sacrificed by cervical dislocation between 1 and 8 days after X ray-irradiation at a dose of 4.0 Gy. Following the fixation of tissue samples including the femoral, the sternal and the humeral bones with Bouin's solution overnight, the bones were immersed in a 1% nitric acid solution for 3 to 4 days for decalcification. Thereafter, the tissue samples were dehydrated with graded ethanol and embedded in paraffin. Five μ m-thick sections were prepared and then stained with hematoxylin and eosine (HE) for histopathological examinations.

RESULTS

The experimental scales and the numbers of survivors as well as mortality 30 days after X ray-irradiation are summarized in Table 1. LEC rats died earlier and had a higher mortality rate at doses of 2.0 Gy or more, while the death of WKAH rats was only noted at doses of 7.4 Gy or more. When the data for the mortality versus X-rays doses shown in Table 1 were applied to probit analysis according to the maximum likelihood method, the $LD_{50/30}$ value was estimated to be 3.0 Gy with a 95% confidence interval of 2.3 to 3.6 Gy for LEC rats, and 7.8 Gy with an interval of 7.4 to 8.1 Gy for WKAH rats, respectively (Fig. 1). Additionally, the upper limit for the confidence interval of the $LD_{50/30}$

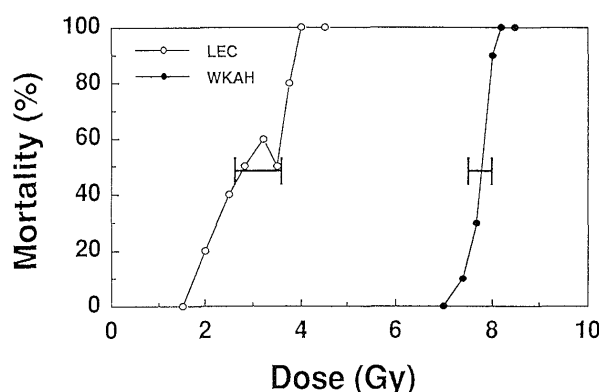


Fig. 1. Probit analysis of the mortality on day 30 after irradiation. Horizontal bars represent 95% confidence intervals of $LD_{50/30}$ values.

Table 1. Summary of the numbers of survivors and the mortality in both LEC and WKAH rats on day 30 after whole-body irradiation at different doses

| Strain | Dose (Gy) | No. of animals exposed | No. of 30-day survivors | 30-day mortality (%) | Surviving time (day) |
|--------|-----------|------------------------|-------------------------|----------------------|-------------------------------|
| LEC | 0 | 10 | 10 | 0.0 | |
| | 1.5 | 5 | 5 | 0.0 | |
| | 2.0 | 5 | 4 | 20.0 | 22 |
| | 2.5 | 5 | 3 | 40.0 | 12,12 |
| | 2.8 | 10 | 5 | 50.0 | 10,10,11,12,12 |
| | 3.2 | 5 | 2 | 60.0 | 10,13,16 |
| | 3.5 | 8 | 4 | 50.0 | 10,11,14,14 |
| | 3.75 | 5 | 1 | 80.0 | 9,10,11,13 |
| | 4.0 | 5 | 0 | 100.0 | 9,10,11,14,17 |
| | 4.5 | 4 | 0 | 100.0 | 11,11,12,12 |
| WKAH | 0 | 10 | 10 | 0.0 | |
| | 7.0 | 5 | 5 | 0.0 | |
| | 7.4 | 10 | 9 | 10.0 | 27 |
| | 7.7 | 10 | 7 | 30.0 | 11,26,28 |
| | 8.0 | 10 | 1 | 90.0 | 12,15,16,17,18 18,19,20,22 |
| | 8.2 | 5 | 5 | 100.0 | 15,16,18,20,21 |
| | 8.5 | 5 | 5 | 100.0 | 10,13,14,18,19 |

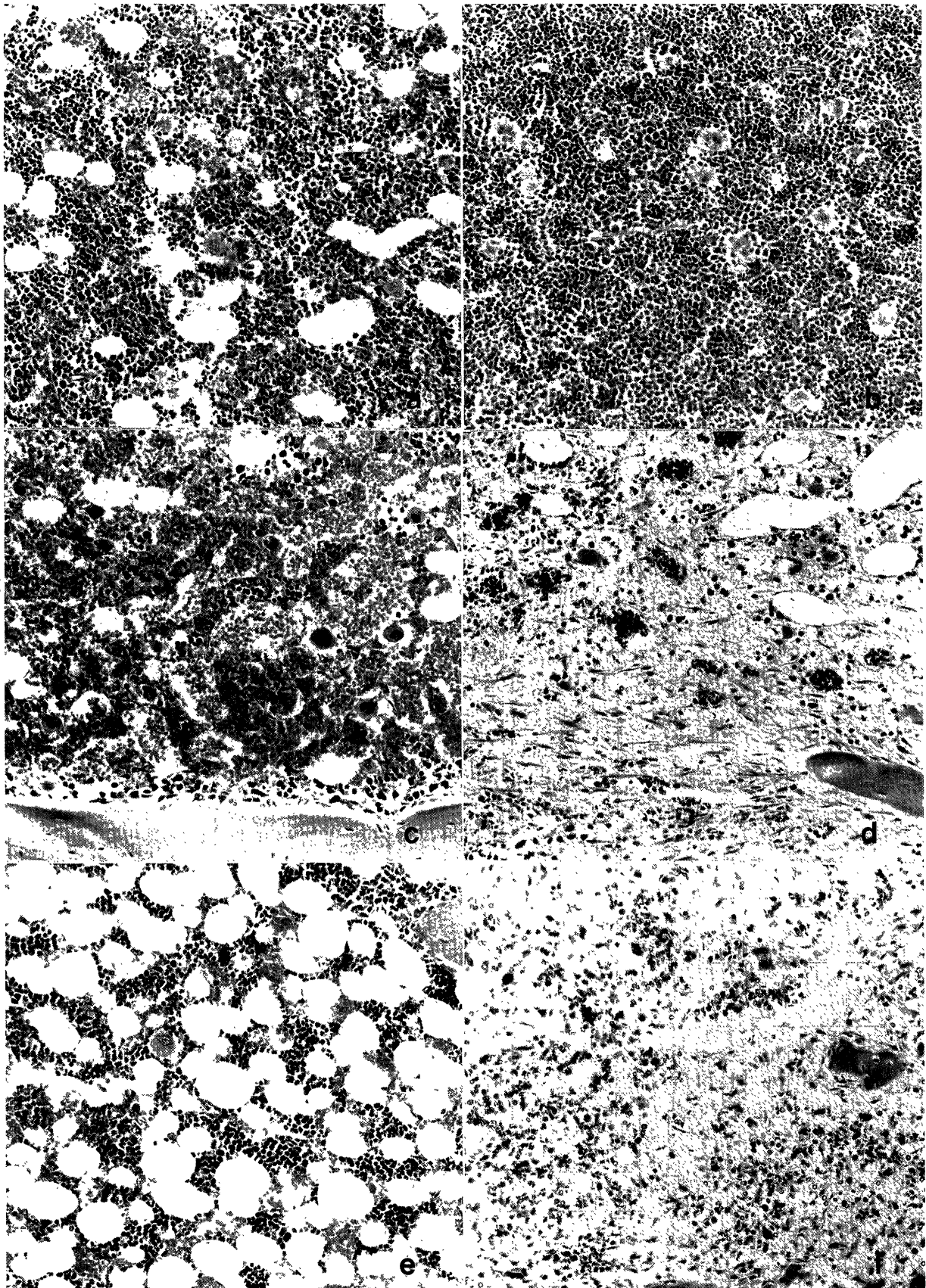


Fig. 2. Histologic figures of bone marrow samples after X ray-irradiation at 4 Gy. a: unirradiated WKAH rat; b: unirradiated LEC rat; c: WKAH rat on day 3 after irradiation; d: LEC rat on day 3 after irradiation; e: WKAH rat at day 8 after irradiation; f: LEC rat at day 8 after irradiation. Hematoxylin-eosin stain after decalcification of diaphyseal regions of femoral bones (magnification $\times 170$).

value calculated in LEC rats was 3.8 Gy lower than the lower limit of confidence interval calculated in WKAH rats. Such distance between confidence intervals of $LD_{50/30}$ values indicated the significant differences in radiosensitivity of LEC rats as compared to WKAH rats.

To see whether the death of irradiated animals was due to acute bone marrow damages, the bone marrow tissues from both LEC and WKAH rats on days 3 and 8 after irradiation at 4.0 Gy were histologically compared to those from unirradiated controls. Unirradiated control of both WKAH and LEC rats showed high cellularity of hemopoietic cells at various differentiation stages throughout the marrow cavities (Fig. 2a and b, respectively). In the cases of WKAH rats on 3-day after irradiation (Fig. 2c), the hemopoietic cells were decreased in number but were distributed either along the bone surfaces or in the sinusoidal spaces, together with many erythrocytes filled in sinusoids. In the bone marrows of LEC rats on 3-day after irradiation (Fig. 2d), both hemopoietic cells and erythrocytes were much more decreased in number, and were replaced by fibrous connective tissues. In the bone marrows of WKAH rats on 8-day after irradiation (Fig. 2e), the number of hemopoietic cells were relatively recovered with increasing adipose cells; in contrast, they were not recovered in LEC rats (Fig. 2f) but instead, fibrous tissues increased. These morphologic findings further indicated that LEC rats were highly sensitive to X ray-irradiation in connection with acute bone-marrow death and recovery.

No severe jaundice was observed in LEC rats between 2 to 3 months of age and no LEC rats died of fulminant hepatitis during the period of the present series of experiments (data not shown).

DISCUSSION

The present data indicated that the death of WKAH rats occurred within 30 days after irradiation at doses more than 7.4 Gy, and the $LD_{50/30}$ value was 7.8 Gy. These results were in good agreement with the reports by other workers [4, 10, 21, 23]. On the other hand, radiation-induced acute death was observed in LEC rats irradiated at much lower doses (2.0 Gy or more) than those of WKAH rats, and their $LD_{50/30}$ value (3.0 Gy) was significantly lower than that of WKAH rats. It is well known that after whole-body irradiation at the $LD_{50/30}$ level, damage to the bone marrow is severe

in all mammalian species including rats [3]. Indeed, a decrease in the number of hemopoietic cells in the bone marrow of both LEC and WKAH rats was observed on 3-day after irradiation at 4 Gy in the present series of experiments. The number of hemopoietic cells was, however, restored in WKAH rats within 8 days after irradiation. Such a decrease in the number of hemopoietic bone marrow cells on day 3 after irradiation, and the recovery of hemopoietic cells on about day 8 after irradiation have also been reported by other workers [3, 6, 7]. In contrast, the number of hemopoietic cells was not restored in the bone marrows of LEC rats even on day 8 after irradiation. The want of earlier recovery of bone marrow hemopoiesis in LEC rats after irradiation would be due to the higher radiosensitivity of hemopoietic stem cells [3].

Although it still remains to be elucidated why the bone marrow cells of LEC rats could be more radiosensitive than those of other strains of rats, the radiosensitivity related to the acute intestinal death of LEC rats was approximately twice as high as that of WKAH rats induced by X ray-irradiation [8]. Preliminary observations showed that the radiosensitivity related to the cell death of the lung fibroblasts isolated from LEC rats was also approximately twice as high as that from WKAH rats and, after whole-body irradiation, the frequency of chromosome aberration in the bone marrow cells of LEC rats was approximately twice as high as that of WKAH rats (data not shown). Therefore, almost all types of stem cells from LEC rats are likely to be more radiosensitive than those of the other strains of rats. In this regard, the radiosensitivity of severe combined immunodeficient (scid) strain (C.B-17) mice has been shown to result in $LD_{50/14}$ values and an incidence of cell death, for example of the bone marrow stem cells, the intestinal crypt cells and the epidermal basal cells approximately twice as high as in BALB/c or parental C.B-17 strain mice [1], and it has further been suggested that scid mutation impairs the repair of the DNA double-strand break [1]. One possible reason for the higher radiosensitivity observed in LEC rats may be the impairment of some processes repairing DNA damage produced by ionizing radiation.

It has recently been demonstrated that copper element accumulates more densely in the liver of LEC rats [12, 13]. Copper can efficiently induce DNA damage *in vitro* in the presence of hydrogen peroxide and reducing agents [11, 25], and the

hydroxyl radical is produced by copper and hydrogen peroxide [11, 19, 25]. The hydroxyl radical is one of the primary reactive species which indirectly induces DNA damage by ionizing radiation [2]. It might also be possible that higher copper accumulation modulates or enhances the radiosensitivity of LEC rats by producing such radicals.

When the hemopoietic stem cells are damaged by X-ray irradiation, the radiation induced death results from bacteremia of endogenous flora [16, 17, 24]. We could not therefore rule out the possibility that the infections of endogenous flora contributing to hemopoietic death may be different in LEC and WKAH rats. Whether endogenous flora affect the radiosensitivity of LEC rats is currently being investigated in SPF rats.

Since the Wistar strains of rats were often used in the field of experimental radiation biology [4, 10, 23], we compared the radiosensitivity of LEC rats with that of inbred Wistar strain (WKAH) rats in the present study, although the genetic backgrounds of LEC and WKAH rats are different. The parental Long-Evans closed colony of LEC rats has expired and is therefore not available now. Although LEA strain, a sibling line of LEC rats, does not develop hereditarily spontaneous fulminant hepatitis [20], there are many differences between the biochemical and immunological characteristics of LEA and LEC rats [12, 13, 15, 20], and preliminary observation showed that the radiosensitivity of LEA rats was significantly lower than that of LEC rats (data not shown). Therefore, in order to clarify the factor which indirectly affects the radiosensitivity of LEC rats and a gene (or genes) which is directly associated with the higher radiosensitivity of LEC rats, a congenic strain of LEC rats with higher radiosensitivity should be established and analyzed. Such a study is now in progress. LEC rats would provide a useful model to help in understanding the mechanism of repair as well as sensitizing agents in radiation-induced damage.

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