Enhanced Radiosensitivity in 1,25-dihydroxyvitamin D3 Deficient Mice

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Radiosensitivity/Knockout mice/1,25-(OH)2D3/Osteogenesis/Hematopoiesis.

To investigate whether impaired osteogenesis resulting from vitamin D deficiency can influence hematopoiesis recovery after radiation, the 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase) gene knockout (KO) mice and wild type (WT) mice were subjected to different doses of gamma ray. The survival rates, peripheral blood cell counts and bone marrow cellularity were studied after irradiation (IR). The survival rates of the KO mice were significantly lower than that of WT mice after 6 or 8 Gy dose of radiation. The recovery of white blood cells in KO mice was significantly delayed compared with that in WT mice after radiation. The red blood cell number in WT mice was observed to increase more than that in KO mice at days 14 and 28 after radiation. The nadir platelet count in KO mice was nearly half of that in WT mice. Dramatically higher bone marrow cell numbers were found in WT mice compared with KO mice. Our findings demonstrate the enhanced radiosensitivity in 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) deficient mice.

INTRODUCTION

More and more available evidence has indicated that the osteogenesis and hematopoiesis are interrelated, although they were traditionally viewed as distinct and unrelated processes.1–5) These observations suggest that osteoblasts are a key component in the regulation of the hematopoietic stem cells (HSCs) niche.6) The osteoblasts can produce many cytokines, including interleukin-6, c-kit ligand and others, which are essential for the survival, renewal, and maturation of HSCs.7–9)

It is well-known that vitamin D plays a major role in the osteogenesis and regulation of mineral homeostasis. Vitamin D can change normal and osteoporotic human osteoblast behavior and stimulate osteoblast differentiation.10–11) Most effects of vitamin D are mainly attributed to the 1,25-dihydroxyvitamin D3 (1,25-(OH)2D3) metabolite.12) 1,25-(OH)2D3 is necessary for base-line bone formation and osteogenesis. In 1,25-(OH)2D3 deficient mice, osteoblast numbers, mineral apposition rate, and bone volume levels were significantly reduced even serum calcium level was normal after rescue diet.13) It has been reported that hematopoiesis was severely damaged in osteoblast deficient mice.14) However, no data are available on whether impaired osteogenesis resulted from vitamin D deficiency can influence hematopoiesis recovery after irradiation (IR). In this study, using a transgenic mice model characterized by undetectable levels of 1,25-(OH)2D3, we provide a novel evidence that abnormal bone metabolism resulted from vitamin D deficiency can influence hematopoiesis recovery after radiation.

MATERIALS AND METHODS

Animals

1α-hydroxylase knockout (KO) mice were kindly provided by Dr. David Goltzman (McGill University, Montreal, Canada). Mice heterozygous for the 1α-hydroxylase allele were mated together to generate pups homozygous for both 1α-hydroxylase null alleles. KO and wild type (WT) pups were identified by PCR using the tail genomic DNA as the template as described previously.15) WT mice in littermates were used as control. All mice are on the C57BL6/J background. To maintain the serum calcium level in KO mice as same as that in WT mice, all of mice were feed with diet Gamma-radiated chow containing 2% calcium, 1.25% phosphorus, 20% lactose, and 2.2 units/g vitamin D. Animals were housed in a temperature and humidity controlled environment and fed chow and water ad libitum. Female WT and KO 7-week-old mice were used in this study. All animal protocols were approved by the animal care committees of the Soochow University.
Radiation
Whole-body radiation was performed with a γ- radiation source (137Cs, Canada). Mice were placed in ventilated Plexiglas cages and irradiated in groups of five mice simultaneously with a dose rate of 0.83 Gy/min at room temperature (23 ± 2°C). The mice were radiated with a total dose of 8, 6 or 4 Gy gamma-rays.

Survival assay
Survival was monitored daily and reported as the percentage of animals surviving 28 days after radiation. Each treatment group consisted of 10 mice. Data were expressed as % survival.

Peripheral blood hematology
Blood was obtained from isoflurane-anesthetized mice via the caudal vena cava using heparinized syringes fitted with 23-gauge needles and was collected in ethylenediaminetetra-acetic acid (EDTA) tubes. Complete blood counts were analyzed using a XFA6000 Hematology Analyzer.

Determination of Serum 1,25(OH)2D3 and 25(OH)D
Serum 1,25(OH)2D3 was extracted with separate extraction columns and measured by radioimmunoassay (BIOSOURCE KIP1921, Invitrogen Co. Ltd, Belgium). Serum 25-hydroxyvitamin D (25(OH)D) concentration was determined by ELISA kits (IDS Ltd, UK).

Determination of bone marrow cellularity
Blood marrow cells were harvested from both femurs into phosphate buffered saline containing 2% fetal calf serum. The number of cells was determined using a hemocytometer and expressed as total cells (×10⁶) / femur.

Statistical analysis
Statistical analysis was performed using the SPSS version 11.0 for windows. Data were presented as mean ± standard deviation (SD). The significance between survival curves was analyzed by Kaplan-Meier survival analysis with log-rank test. One way analysis of variance (ANOVA) was adopted to evaluate differences between groups. A difference was considered to be statistically significant when p < 0.05.

RESULTS AND DISCUSSIONS
The synthesis of 1,25(OH)2D3 from its precursor 25-hydroxyvitamin D is catalyzed by the mitochondrial cytochrome P450 enzyme 25-hydroxyvitamin D-1α-hydroxylase (1α-hydroxylase). There are no detectable 1,25(OH)2D3 in mice targeted ablation of the 1α-hydroxylase gene. This result was confirmed in our study both before and after radiation (Table 1). 25-hydroxyvitamin D level in serum was not significantly different between KO and WT mice before or after radiation (Fig. 1). So, this transgenic mouse is a perfect model for investigating the effects of 1,25(OH)2D3 deficiency on radiosensitivity.

The major finding of the present study was that KO mice exhibit increased mortality after exposure to ionizing radiation. Mice exposed to a dose of 8 Gy were followed for 28 days. None of the KO mice survived after 16 days post-radiation. Of the WT mice, 75% survived (p < 0.01 compared to the KO mice) 28 days post-radiation (Fig. 1A). Consequently, a lower dose of radiation was adopted on mice, 50% mortality in the KO mice was observed on day 18 after treatment with 6 Gy, while no mortality in the wild

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<tr>
<th>25-(OH)D(ng/ml)</th>
<th>1,25(OH)2D3 (pg/ml)</th>
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<tr>
<td>before IR</td>
<td>3 days after IR</td>
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<td>before IR</td>
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<td>KO 25 ± 3.8</td>
<td>27 ± 2.3</td>
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<tr>
<td>WT 23 ± 2.1</td>
<td>26 ± 3.6</td>
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Table 1. Serum level of 25-(OH)D and 1,25(OH)2D3 in mice

Fig. 1. The survival rates of mice radiated with 8 Gy (A) or 6 Gy (B) gamma-rays. 1α-hydroxylase knockout (KO) and wild type (WT) mice were exposed to gammy ray of 8 Gy (A) or 6 Gy (B). Each treatment group consists of 10 mice. Data were expressed as % survival. The significance of survival rate between groups exposed to same dose was analyzed by Kaplan-Meier survival analysis with log-rank test.
Radiosensitivity in 1α(OH)ase Knockout Mice

It is generally accepted that damage in hematopoietic system plays a critical role in radiation-induced death. Radiation-induced damage is thought to be resulted primarily from the effects of radiation on hematopoietic cells. To investigate the cause of death of the irradiated KO mice, peripheral blood cell and bone marrow cell numbers in both KO and WT mice at 3 and 7 days after 8 Gy irradiation were detected. Circulating WBC and bone marrow cell counts were significantly different between the KO and WT mice at day 3 or day 7 post-irradiation (Fig. 2A, 2D). On day 7 after irradiation, WT mice exhibited elevated WBC numbers while WBC numbers in KO mice still remained low (Fig. 2A). RBC and PLT counts were significantly higher in WT mice than in KO mice on day 7 post-irradiation, although significant difference was not detected on day 3 post-irradiation (Fig. 2B, 2C).

To further investigate the different effects of radiation on WT and KO mice, mice were exposed to lower dose radiation. White blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts in peripheral blood are shown in Fig. 3. After 4 Gy of radiation, circulating WBCs, RBCs and PLTs markedly decreased in both WT and KO mice because of hematopoietic injuries caused by radiation. Compared with WT mice, there are much lower peripheral blood cell counts in KO mice through all time point after irradiation. WBC counts decreased rapidly and the level of WBC in the KO mice reached a lower nadir. The rate of recovery in WBC was nearly identical for the WT and KO mice. However, the WBC number was not completely recovered in KO mice. On day 28 after radiation, WBC counts in WT mice returned to normal, as compared with the values of 71.1% in KO mice (Fig. 3A). RBC numbers decreased slowly and reached nadir at day 14 after radiation. Significantly increased RBCs in WT mice compared with KO mice were observed at day 14 and 28 after radiation (p < 0.01, Fig. 3B). Similar to RBC, PLT numbers reached nadir at day 14 after radiation. The median nadir of PLTs (325 × 10⁹ cells/L) in KO mice were much lower than that in WT mice (422 × 10⁹ cells/L) (p < 0.05, Fig. 3C). Consistent with these observations, we found a reduction in bone marrow cellularity in KO mice on 28 day after radiation while bone marrow cellularity in WT mice was returned to normal at same time point (Fig. 3D).

The role of vitamin D in hematopoiesis has been reported. However, our study is the first report on the increased radiosensitivity in 1,25-dihydroxyvitamin D₃ deficient mice. The exact mechanisms underlying the increased radiosensitivity in KO mice are not clear yet. It is confirmed that osteoblast numbers, mineral apposition rate and bone

Fig. 2. Peripheral blood cell count and bone marrow cellularity in radiated mice. Peripheral blood cell count was analyzed using a XFA6000 Hematology Analyzer in WT and KO mice after 8 Gy of radiation. (A) WBC, (B) RBC, (C) PLT and (D) bone marrow cell counts were shown as mean ± SD (n = 5). One-way ANOVA was used to evaluate difference between groups. a, p < 0.05, b, p < 0.01 compared with KO mice at the same time point.
volume levels in 1,25-(OH)₂D₃ deficient mice were significantly reduced even serum calcium level was normal after rescue diet. Because the interplay between osteogenesis and hematopoiesis has been reported by several papers, it is speculated that impaired osteogenesis resulted from 1,25-(OH)₂D₃ deficiency may be contributed to the slow hematopoiesis recovery after radiation.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 3. Peripheral blood cell count and bone marrow cellularity in radiated mice. Peripheral blood cell count was analyzed using a XFA6000 Hematology Analyzer in WT and KO mice after 4 Gy radiation. (A) WBC, (B) RBC and (C) PLT counts were shown as mean ± SD (n = 5). On 28 day after 4 Gy radiation, bone marrow cellularity was determined using a hemocytometer and expressed as total cells (×10⁶) / femur counted in WT and KO mice (D). Data were shown as mean ± SD (n = 5). One-way ANOVA was adopted to evaluate differences between groups. a, p < 0.05, b, p < 0.01 compared with same time point KO mice.
Radiosensitivity in 1α(OH)ase Knockout Mice


