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THE LATE EFFECTS ON BONE MARROWS IN MICE AFTER TOTAL BODY IRRADIATION BY P(35) BE FAST NEUTRONS AND γ RAYS

Sun Yan 孙艳 Han Shukui 韩树奎 Xu Bo 徐博 Zhang Shuhui 张淑惠
Tang Jinghua* 唐景华 Chang Weike* 常维克 Shen Wenjiang 申文江

Radiotherapy Department, Beijing Institute for Cancer Research, Beijing 100034
**Institute of High Energy Physics, Academia Sinica, Beijing 100039*

Purpose: To understand the late effects on bone marrow after widefield or total body irrdiation with various types of radiation.

Materials and Methods: Total body irradiation with single dose of P(35) Be fast Neutrons and γ rays respectively have been used in this study. Kunming strain mice were irradiated by fast neutrons produced by bombardment of beryllium target with 35MeV protons and the dose rate was 0.12 to 0.14 Gy/min. Nine dose levels were used in fast neutrons irradiation from 0.20 to 3.50Gy. The dose rate of ^{60}Co γ rays was 0.60Gy/min and irradiation was in the range of 0.25 to 9.00Gy. Sixteen dose points were studied. All animals without anesthesia were irradiated whole body with single doses by fast neutrons and γ rays respectively. One group without irradiation was regarded as control group. 90 days after irradiation all animals were sacrificed. The nucleated cells of bone marrow and the peripheral blood cells including WBC and lymphocytes were counted.

Results: Our study shows that the number of nucleated cells of bone marrow in both fast neutrons and γ ray groups decreased with increase of the doses. There are significant differences between irradiated groups and zero line (control group) and the slopes are -1.41 ± 0.55 (p=0.038) and -0.98 ± 0. 24(p= 0.0015) for fast neutrons and γ rays respectively. There is no significant difference between the two kinds of radiation (P>0.05). The late effects on peripheral blood cells (WBC and lymphocytes) in mice after irradiation with single doses of neutrons and γ rays were significantly lower than unirradiated group (P< 0.05). However, the dependence of the values of peripheral blood cells on doses is not found and there are no significant differences between neutrons and γ rays groups.

Conclusions: Total body irradiation with neutrons or γ rays can suppress marrow in mice in the long-term, and is more obvious with increase of doses. There is no significant difference between neutrons and γ rays for the late effects studied.

Key words: Fast neutrons, Bone marow, Irradiation

The bone marrow is a dose-limiting cell renewal tissue for wide-field irradiation, in spite of both early and late period after the radiotherapy. The damage of the hematopoietic stem cell and microenvironment of the bone marrow can be found after irradiation and the long-term myelosuppression can also be resulted in when wide-field irradiation is used. It's very important for further clinical therapy to understand the late effects on bone marrows after wide-field or total body irradiation. In this paper the late effects on bone marrows in mice after total body irradiation with single dose of p(35) Be fast neutrons and γ rays has been studied.

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MATERIALS AND METHODS

Animals

Kunming strain mice, which were equal numbers of male and female, were used in our experiments. They were 6 — 8 weeks of age and 20 — 22 gram of weight. There were 10 to 15 mice in per experimental group.

Fast Neutrons Irradiation

The fast neutrons were produced by bombardment of beryllium target with 35 MeV protons coming from proton linear accelerator in Institute of High Energy Physics, Academia Sinica. The distance from the target to skin surface is 143 cm. Nine dose levels in fast neutrons experiment range from 0.20 to 3.50 nGy. The dose rate is 0.12 to 0.14 Gy/min. The γ rays dose contribution in neutron beams was not considered.

^{60}Co γ Rays Irradiation

^{60}Co γ rays were provided by Beijing Institute for Cancer Research. The dose rate was 0.60 Gy/min. Sixteen dose points ranging from 0.25 to 9.00 Gy were studied. Animals were laid within 80% dimension of irradiation field in order to obtain uniform dose distribution. The ion chamber with balance tissue was placed among mice to monitor the dose received by mice.

Experiment Methods

All animals without anaesthesia were irradiated whole body with single doses in the both case of fast neutrons and γ rays. One group of mice without irradiation was regarded as control group. All animals were sacrificed by encephalospinal amputation on 90 days after irradiation. The nucleated calls of bone marrow were studied with left femurs of mice. The peripheral blood cells including WBC and lymphocytes were counted as well.

Statistical Methods

The survival curves of nucleated cells of bone marrow were drawn up using linear equation model. The *t* test and F test were used for statistical significance in this experiment.

RESULTS

Number of Nucleated Cells of Bone Marrow

Our study shows that the number of nucleated cells of bone marrow in mice are decreasing with increasing of the both doses of fast neutrons and γ rays. There are significant differences between irradiated groups and control group. The p values are 0. 038 and 0.0015 for fast neutrons and γ rays respectively. The slopes of the survival curves are -1.41±0.55 and -0.98±0.24 for fast neutrons and γ rays respectively, which are showed in Figure 1. However, there is not a significant diffefence between above two irradiated groups (P >0.05).

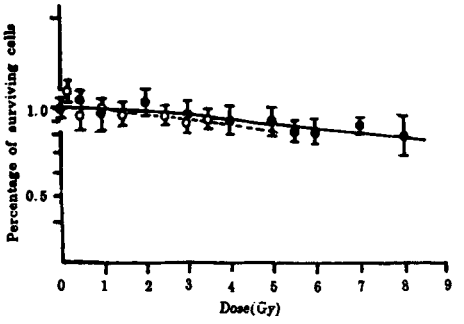

Fig 1. The survival curves for late effects of the nucleated cells on bone marrow in mice after irradiation with single doses of p(35) Be fast neutrons and ^{60}Co-γ rays. Open circles: P(35)Be fast neutrons; Closed circles: ^{60}Co-γ rays. The error bars represent the mean and SE.

Changes of peripheral Blood Cells

The late effects on peripheral blood cells (WBC and lympocytes) in mice after whole body irradiation with single doses of fast neutrons and γ rays were also observed. The changes following irradiation were showed in Figure 2. The values of the peripheral blood cells of the irradiated groups including both fast neutrons and γ rays were significantly lower than the unirradiated group (P<0.05). However, the dependence of the values of peripheral blood cells on doses is not found and there are not significant differences between fast neutrons and γ rays groups (Table 1).

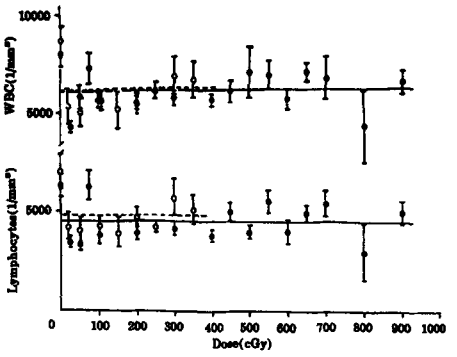

Fig 2. The survival curves for late effects on peripheral blood cells including WBC (upper) and lymphocytes (low) in mice after irradiation with single doses of P(35) Be fast neutrons; Closed circles: ^{60}Co-γ rays. The error bars represent the mean and SE.

Table 1. The late effects of irradiation with fast neutrons and γ rays on the peripheral cells in mice

The peripheral cells	Fast neutrons	γ rays	*P* values
WBC	0.579±3.27	0.128±0.95	>0.05
Lymphocytes	$8.19\times10^{-4}\pm2.98$	$-3.93\times10^{-4}\pm0.93$	>0.05

DISCUSSION

The bone marrow is a dose-limiting tissue for irradiation. It is extremely sensitive to degree of any irradiation dose. Irradiation damages stem cells and the microenvironment which has been well reviewed by Mauch.[1]

Early and late myelosuppression can be resulted in by wide-field or whole body irradiation with fast neutrons and ^{60}Co γ rays. Early effect of the bone marrow appears decreasing in number of nucleated cells with increase of the doses. The early effects of fast neutrons and γ rays on bone marrow in mice are different. There is a significant difference between the fast neutrons and γ rays and RBE value is 2.13 ± 0.18.[2]

The bone marrow stroma damage is more important in the late period of chronic radiatiion injury. Dexter technique is the most widely used assay of stromal function.[3] The capacity for bone marrow repair is great *in vivo*. The femoral bone marrow of rats have only a transient effect on stromal function after femur local irradiation with doses of 10 Gy, 250kvp X rays. However, single doses of 20 — 100 Gy result in delayed destruction of sinusoids, then hematopoiesis losts.

There is not significant difference between these two kinds of rays within the dose range we used in this experiment. It suggests the capacity for bone marrow repair in mice is great. There is not balance between changes of the peripheral blood cells and the nucleated cells of bone marrow.

Moreover, some cytokines play an important role in radioprotection, such as G-CSF or IL-1 that have been found to improve survival and facilitate hematopoietic recovery.[4,5] So, it deserves to further study in radiotherapy, especially wide-field irradiation.

It is important to understand the effects of various types of radiation on bone marrow damage and protect, and reduce those toxic effects for clinical radiotherapy and chemotherapy of tumor.

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