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Onderwerp: Effects of low-dose radiation on tumor growth, erythrocyte immune function and SOD activity in tumor-bearing mice

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Effects of low-dose radiation on tumor growth, erythrocyte immune function and SOD activity in tumor-bearing mice

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Background Activating on mammalian and human body LDR is thought to induce adaptive response, enhance immune function and increase anti-tumor ability. This study was designed to assess the effect of low-dose radiation on tumor growth and on erythrocyte immune function and superoxide dismutase (SOD) activity in tumor-bearing mice.
Methods Male Kunming mice were subcutaneously implanted with S180 sarcoma cells in the right inguen to create an experimental in situ animal model. Six hours before implantation, the mice were given 75 mGy X-ray radiation, over the body. Tumor size was observed 5 days later while tumor volume was calculated every other day, allowing for the creation of a graph depicting tumor growth. Fifteen days after implantation, the mice were killed to measure tumor weight and observe the necrotic areas and the location of tumor-infiltrating lymphocytes (TILs). Erythrocyte immune function and SOD activity were also determined.
Results Mice pre-exposed to low-dose radiation had a lower tumor formation rate than did those receiving no radiation ($P<0.05$). Tumor growth was significantly lower in the mice pre-exposed to low-dose radiation; after 15 days, the average tumor weight in the mice pre-exposed to low-dose radiation was also lower ($P<0.05$). Areas of tumor necrosis and infiltration of TILs were larger in the low-dose radiation group than in the non-radiation group. Erythrocyte immune function and SOD activity were higher in the low-dose radiation group than in the non-radiation group ($P<0.05$).
Conclusion Low-dose radiation can markedly increase the anti-tumor ability of an organism and improve erythrocyte immune function and red blood cell SOD activity as well, suggesting that low-dose radiation might be useful in the clinical treatment of cancer.

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Radiotherapy and chemotherapy are the primary methods for the treatment of malignant tumors; but both treatments can lead to injury because they inhibit immune function, and inevitably influences clinical results. Some patients with malignant tumors die of complications because of inhibition of immune function. How to boost these patients' immune system has become an important topic of research. Reports have indicated that low-dose radiation has an excitatory effect on lymphocyte immune function, [1,2] but few on the effects of low-dose

radiation on erythrocyte immune function. This study was designed to assess the effects of low-dose radiation on tumor growth and on erythrocyte immune function and superoxide dismutase (SOD) activity in tumor-bearing mice. The results may provide theoretical evidence for the clinical use of low-dose radiation.

METHODS

Animals

Male Kunming mice weighing 20±2 g and aged 4 to 6 weeks were purchased from the Qingdao Pharmaceutical and Testing Institution, Qingdao, China. They were randomly divided into three groups: normal control; low-dose radiation; and non-radiation. Mice in the last two groups were implanted with S180 sarcoma cells and kept under mormal conditions and free accessible to water and food.

Radiation conditions

A Beijing F34-1 deep X-ray machine was used, with a voltage of 220 kV, an electric current of 10mA, and a filter composed of 0.5 mm Cu + 1.0 mm Al. The mice in the second group were given whole body radiation with a total dosage of 75 mGy given at 12.5 mGy/min six hours before the implantation of S180 sarcoma cells.

Implantation of S180 sarcoma cells

S180 sarcoma cells purchased from the Chinese Medical Science Institute were allowed to replicate for two generations in mouse abdomens. Cell solution during the logarithmic growth phase had a density of 1×10 (7) cells/ml. All the experimental mice except those in the normal control group were then implanted subcutaneously in the right inguen with 0.1 ml of S180 sarcoma cells [total 1×10 (6) cells] .

Assessment of tumor growth

Beginning 5 days after the implantation of the S180 sarcoma cells, tumor size was assessed in the mice every other day by measuring the maximum horizontal diameter (a) and the maximum vertical diameter (b) twice with a slide gauge. The tumor's volume (V) was then computed using the formula: V=1/2ab2. [1] Finally, graphs depicting tumor growth were produced.

Examination of tumor biopsy specimens

Fifteen days after implantation, the mice were sacrificed for the removal of tumors which were weighed subcutaneous fat was separated. Each tumor was then fixed in 10% formalin for several days, sliced, and stained with HE. The necrotic areas of the tumors and the regions of lymphocyte invasion were observed.

Determination of erythrocyte immune function

Fifteen days after implantation, blood was taken from the venous sinus of the right eye of each mouse. The densities of red blood cell surface C3b receptors (RBC-C3bRR) and red blood cell immune compounds (RBC-IcR) were determined. [2] The mice in the normal control group were examined following these procedures. Under a high diplo-microscope(10×100), red blood cell having two or more than two yeasts was regarded positive. Two hundred red blood cells were counted, and the percentage of positive red blood cells was determined.

Determination of erythrocyte SOD activity

Fifteen days after implantation, blood was taken from the venous sinus of the right eye of each mouse. One nitrite salt unit was the corresponding quality of SOD inhibitory rate up to 50% for every 1 ml response liquid. The unit of enzyme activity was based on NU/gHb. [3] The mice in the normal control group were examined using the same procedures.

Statistical analysis

All values were expressed as mean±standard deviation. Student's t test was used to compare the two groups. The Student-Neuman-Keuls procedure was used to analyze multi-group data.

RESULTS

Anti-tumor effects of low-dose radiation

Inhibition effects on tumor occurrence

Mice pre-exposed to low-dose radiation had a lower rate of tumor occurrence (71.43%) than did those receiving no radiation (97.37%, $P<0.05$).

Inhibition effect on tumor growth

Beginning on the 5th day after implantation of S180 sarcoma cells, tumor sizes were calculated every other day (Table 1). The tumor sizes of the mice pre-exposed to low-dose radiation were significantly smaller than those of mice receiving no radiation on the 7th day and, especially, from the 9th day onward.

Examiniation of tumor biopsy specimens
On the 15th day after implantation, the average weight of the tumors in the mice pre-exposed to low-dose radiation was (0.743±0.210) g, significantly lower than that in the mice receiving no radiation [(1.330±0.298) g] .

Microscopically, tumors from the mice receiving low-dose radiation showed a larger areas of necrosis and more significantly invaded lymphocytes than did those from the mice receiving radiation (Figs.1-4).

Effects of low-dose radiation on erythrocyte immune function
RBC-C3bRR density in the normal control group was higher than that in the group receiving no radiation ($P<0.05$), and RBC-IcR density was lower ($P<0.05$). However, both RBC-C3bRR and RBC-IcR densities in the mice pre-exposed to low-dose radiation were not different from those than in the normal control group ($P>0.05$). Furthermore, RBC-C3bRR density was higher in the mice receiving low-dose radiation than in the mice receiving no radiation ($P<0.05$), but RBC-IcR density was lower ($P<0.05$, Table 2).

Effects of low-dose radiation on erythrocyte SOD (E-SOD) activity
E-SOD activity in the normal control group was higher than that in the group receiving no radiation ($P<0.05$), but it was not different from that in the low-dose radiation group ($P>0.05$). The E-SOD activity was higher in the low-dose radiation group than in the group receiving no radiation ($P<0.05$, Table 3).

DISCUSSION

Low-dose radiation can decrease the extent of and inhibit the occurrence of tumors by activating the immune system. [1,2] Our study showed that mice pre-exposed to low-dose radiation (75 mGy whole-body X-ray radiation) have a better ability to inhibit tumor development and growth than do mice receiving no radiation. This conclusion accords with previous reports. [4]

Horst's study [5] showed that TILs are 50 to 100 times more effective than lymphokine-activated killer (LAK) cells, so TILs can serve as an index of the body's immune function. This study showed that low-dose radiation can enlarge the area of tumor necrosis and lead to greater infiltration of TILs into tumor tissue. These results indicate that low-dose radiation can activate the immune system and improve the anti-tumor capability of the murine immune system.

For a long time, it has been thought that white blood cells (WBCs) play very important roles in the body's immune system, whereas red blood cells (RBCs) only fanction for transmition of oxygen and carbon dioxide. In fact, RBCs can adhere to tumor cells by means of surface C3b receptors (C3bRs), which lie on the membrane surface of RBCs. The RBC complexes can then be engulfed by macrophages. [6] In the peripheral blood, RBCs are about 10 (3) times more common than WBCs and can form globular immune complexes more easily, enhancing macrophage function. RBC C3bRs may be blocked following the inhibition of the body's immune function and the construction of globular immune complexes. Thus, a large number of these immune complexes may build up in tissues and organs, leading to tissue or organ injury. It was reported that the immune function of erythrocytes is positively correlated with that of lymphocytes. [7] Erythrocyte immune function can be measured using only a small sample of blood, so erythrocyte immune function is more convenient to assay clinically than lymphocyte immune function.

In this study, murine erythrocyte immune function was inhibited after tumor cell implantation, resulting in decreased RBC-C3bR and RBC-C3bRR activity and a build-up of globular immune complexes and RBC-IcRs. After low-dose radiation, erythrocyte immune function in mice with implanted tumors did not significantly differ from that in normal mice, suggesting that low-dose radiation has a hormesis on erythrocyte immune function. Whether inhibition of tumors by low-dose radiation is related to the improvement of erythrocyte immune function requires further investigation.

High-dose radiation does harm to the body via its effects on atoms and molecules, especially water molecules, leading to the production of free radicals, which play a very important role in radiation injury to DNA. Therefore, a decrease in free radicals and/or the enhancement of the anti-oxidization system may alleviate the injury caused by radiation.

Free radicals can be oxidized by SOD and transformed into H2O, which is not poisonous to the body. Normally, the production and the clearance of SOD remains in dynamic balance. It has been reported that the SOD level in patients with malignant tumors is significantly lower than normal and is positively associated with erythrocyte immune function. [8] Consequently, the determination of RBC SOD activity can be a quantitative index of the body's ability to combat the effects of free radicals.

In this study, SOD activity decreased significantly and the level of free radicals increased in parallel with tumor growth in mice implanted with tumor cells; but after low-dose radiation, SOD activity in these mice increased. The hormesis of low-dose radiation on the SOD activity of erythrocytes may be one of the mechanisms leading to the enhancement of erythrocyte immune function.

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