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Effects of low dose pre-irradiation on hepatic damage and genetic material damage caused by cyclophosphamide

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Abstract. – OBJECTIVE: Cyclophosphamide (CTX) can attack tumour cells, but can also damage the other cells and microstructures of an organism at different levels, such as haematopoietic cells, liver cells, peripheral lymphocyte DNA, and genetic materials. Low dose radiation (LDR) can induce general adaptation reaction. In this study, we explore the effects of low dose radiation on hepatic damage and genetic material damage caused by CTX.

MATERIALS AND METHODS: Mice were implanted subcutaneously with S180 cells in the left groin (control group excluded). On days 8 and 11, mice of the LDR and LDR+CTX groups were given 75 mGy of whole-body γ-irradiation; whereas mice of the CTX and LDR+CTX groups were injected intraperitoneally with 3.0 mg of CTX. All mice were sacrificed on day 13. DNA damage of the peripheral lymphocytes, alanine aminotransferase (ALT) activity, total protein (TP), albumin (ALB) of the plasma, malonyl-dialdheyde (MDA) content, superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX) activity of the hepatic homogenate, and micronucleus frequency (MNF) of polychromatoerythrocytes in the bone marrow were analysed.

RESULTS: The control group had the lowest MDA content and the highest SOD and GSH-PX activity, whereas the CTX group had the highest MDA content and the lowest SOD and GSH-PX activity. Compared with the CTX group, the MDA content decreased significantly ($p < 0.01$) and the SOD and GSH-PX activity increased significantly ($p < 0.05$) in the LDR+CTX group. TP and ALB in control group were higher than that of the other groups. Compared with the sham-irradiated group, TP and ALB in the LDR group elevated significantly ($p < 0.05$). The control group had the lightest DNA damage, whereas the CTX group had the severest. DNA damage in LDR+CTX group was much lighter compared with that of the CTX group ($p < 0.05$). MNF in the CTX group increased significantly compared with the control and the sham-irradiated groups ($p < 0.01$). Compared with the CTX group, MNF in LDR+CTX group had a tendency of decline, but without statistical significance ($p > 0.05$).

CONCLUSIONS: Pre-chemotherapeutic LDR can induce the activities of anti-oxidative enzymes and promote the elimination of free radicles to alleviate the damaging effects of oxidative stress to hepatic tissue caused by high-dose CTX. At the same time, LDR has no obvious effect on the ALT activity of plasma, but may have protective effect on the protein synthesis function of the liver. High-dose CTX chemotherapy can cause DNA damage of peripheral lymphocytes; however, LDR before chemotherapy may have certain protective effect on DNA damage. Moreover, CTX has potent mutagenic effect; however, LDR may have no protective effect against the genetic toxicity of CTX chemotherapy.

Key Words:
Low dose irradiation, Cyclophosphamide, Hepatic damage, DNA damage, Micronucleus.

Introduction

Experimental studies have proven that low doses of ionising radiation induce various cancer types with various effects, including radioadaptive response, all activation of immune function, and all enhancement of resistance to high-dose radiation in the initial slope of the cell survival curve[1, 2]. In recent years, studies on the effects of low dose radiation (LDR) have attracted the attention of scientists in radiation research[1,3]. Joiner et al[4] have pointed out that low doses of ionising radiation demonstrated a unique radiobiologic phenomenon, which is an initial phase of the hyperradiosensitivity (HRS) of LDR. Radiation-induced cell cycle arrest provides an opportunity for cells to repair DNA damage before entering the mitotic phase[5]. The discovery that HRS does not stimulate cellular repair mechanisms, such as those seen at higher doses, but provides a plausible explanation on why there is no induction of radio resistance with HRS, as measured *in vitro*[6].

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Given their complex genetic composition, many tumours tend to demonstrate resistance to therapy at the outset or during initial therapy[6]. To take advantage of the benefits of HRS radiation dose in the clinical setting, one logical alternative to exploit the enhanced cell killing at LDR (at which HRS is observed) is to combine with systemic chemotherapy.

We recently reported that LDR can markedly improve the tumour therapeutic efficacy of chemotherapy through the reduction of chemotherapy damage on the immune system and stimulation of the antitumor immune reaction in tumour-bearing mice. The immune depression caused by a larger dose of X-rays can be reduced to a certain extent by low dose pre-irradiation[7]. LDR combined with chemotherapy also improved the immune indexes in tumour-bearing mice. The tumour diameter in the mice treated with cyclophosphamide (Cytoxan, CTX) and LDR at different times after treatment was much smaller than that of the mice with CTX chemotherapy alone. The cytotoxic activities of natural killer, lymphokine-activated killer, and specific cytotoxic T lymphocytes were significantly increased compared with those of the mice with CTX chemotherapy alone. Swatee et al[8] have also found that low-dose ionising radiation delivered in fractionated form (ultrafractionation) acts synergistically with chemotherapy *in vitro*. Preclinical data have indicated that low-dose ionising radiation provides optimal cell killing *in vitro* when combined with chemotherapy.

LDR can induce general adaptation reaction, indicating that LDR can induce DNA and chromosome damage, gene mutation, cell death, immune function restraint, and tumourigenesis rate[9,10]. Such adaptation reaction induced by LDR showed resistance to large dose chemotherapy. CTX is a common cytotoxicity chemotherapeutics in clinics, and can do certain damage to haematopoietic system and genetic materials. This research further investigates the effect of LDR on hepatic damage, DNA damage in peripheral lymphocytes, and genetic material damage induced by high dosage of CTX).

Materials and Methods

Objects

Clearing Kunming strain mice (male, weighting 20±2 g, aged 4-6 weeks) were provided by Qingdao Experimental Animal and Animal Experiment Centre. The animals were randomly divided into five groups: control group, cancer-bearing group (sham-irradiated group); low dose irradiation group (LDR group), CTX chemotherapy group (CTX group); and low dose irradiation combined with chemotherapy group (LDR+CTX group). All mice were raised routinely with unlimited water and food, and accommodated for one week. S_{180} sarcoma cells were generated in the abdomen of the mice for 10 days. Abdominal dropsy was drawn off. The cell suspension in the logarithmic growth phase was made (8×10^7 cells/mL) routinely[2]. The mice, except those in control group, were subcutaneously implanted with 8×10^6 S_{180} sarcoma cells (0.1 mL) in the left inguen.

Radiation Conditions

Once total body radiation with ^{60}Coγ-ray, the mice were placed in a self-made carton, with a 30 cm water mould in the middle to filter the radiation. The radiation distance was 209.5 cm. The dosage rate was 11.14 mGy/min, and the total absorbed dosage was 75 mGy[12].

Irradiation of Tumour-bearing Mice and Chemotherapy

Eight days after implantation, maximum horizontal diameter *a* (cm) and vertical diameter *b* (cm) were measured twice, respectively, with a slide gaud and obtained the averages. The mice with either too large (ab >1.5 cm^2) or too small (ab <0.40 cm^2) tumours were excluded. The average tumour sizes were calculated according to the following formula: $V = (1/2)\ ab^2$ [11] and[12]. If there was no difference in the sizes of tumours among all the groups, the mice in the LDR and LDR+CTX groups were given low dose γ-irradiation, and then 3.0 mg of CTX was injected into the abdominal cavity of both the CTX and LDR+CTX groups after 30 hours (on day 9). This process was repeated on days 11 and 12. All mice were sacrificed on the 13th day.

Example Collection

Collecting blood from fossa orbitalis[20]: the mouse was fixed in place, pushed its head slightly to eyeball evagination, and the eyeballs were removed with ophthalmology nipper. The blood was dropped into an EDTA pipe, mixed immediately, and preserved at 4 °C. After centrifugation for 10 min at 3500 r/min, blood plasma was collected for detection of peripheral blood lymphocyte DNA. According to Wang et al[15], livers were rapidly removed after the execution of the mice by cutting

the cervical vertebra, made into 10% (W/V) liver tissue homogenate in condition of 4 °C., Supernatant fluid was then collected, centrifuged for 10 min at 3500 r/min, preserved at –20 °C, and the detected after one week. Obtaining bone marrow: the mice were sacrificed by cutting the cervical vertebra. The skin of the inguinal region of the right hind limb was clipped and the muscle was separated to expose the femoral bone. Bone marrow was obtained for the detection of the micronucleus frequency (MNF) of polychromatoerythrocytes (PCE).

Detection items

Alanine Aminotransferase (ALT) and Protein Concentration in Plasma were Measured

ALT, total protein (TP), and albumin (ALB) were analyzed with an Olympus-400 automatic biochemistry analyzer (Tokjo, Japan).

Assay of Superoxide Dismutase (SOD)[12] Activity

Superoxide anion free radical, produced from xanthine and xanthine oxidase reaction system, oxidised hydroxylamine to form nitrites, which appeared violet in the chromogenic reaction, and whose absorbency can be measured by visible spectrophotometer. SOD in the samples suppressed superoxide anion free radical with specificity and reduced the production of nitrites; hence, the measuring tube had lower absorbance value than the control tube. One SOD activity unit in a tissue is equal to the SOD amount when the SOD inhibition rate is 50% per mg protein in 1 mL of reaction liquid. Calculation formula[13]: SOD activity in tissue homogenate (U/mgprot) = (absorbance of the control tube – absorbance of the measuring tube) / absorbance of the control tube / 50% × (volume of the total reaction liquid / sampling volume) / protein content in the tissue.

Assay of Glutathione Peroxidase[17] (GSH-PX) activity

GSH-PX can prompt hydrogen peroxide (H_2O_2) to react with reduced glutathione (GSH) to form oxidative glutathione (GSSG). The velocity of the GSH-PX enzymatic reaction represents its activity. The activity of the enzyme can be obtained by measuring the GSH consumption in this enzymatic reaction. One enzyme activity unit is equal to the GSH-PX amount that can reduce GSH concentration by 1 μmol/L, while deducting the effect of nonenzymatic reaction. Calculation formula: GSH-PX (activity unit) = (absorbance of nonenzymatic tube-absorbance of the enzymatic tube) / (absorbance of standard tube-absorbance of the blank tube) × concentration of the standard tube (20 μmol/L) × dilution multiple (5) / reaction time ÷ protein content in tissue.

Measurement of Malonyl-dialdheyde[14] (MDA) Activity

TBA method: MDA can induce the condensation of the red product with thiobarbituric acid (TBA), which has the highest absorption pick at the 532 nm wavelength place.

Calculation formula = (absorbance of the tube – absorbance of the blank measuring tube) ÷ (absorbance of the standard tube – absorbance of blank standard tube) × standard sample concentration (10 nmol/mL) / protein content.

All assay kits were supplied by Nanjing Jiancheng Bioengineering Institute, and the instrument is 721 model grating spectrophotometry (The Third Analytical Instrument Factory in Shanghai).

Detection and Analysis of DNA Damage[21]

Anticoagulated blood (35 μL) was obtained with a micropipette, and lymphocytes were obtained by lymphocyte isolation fluid abstracting and centrifuging. Approximately 85 μL of 1% drown standard gelose was obtained, dropped on a glass slide, quickly covered with coverslip, and then placed in a freezer at 4 °C for 5 min to 10 min. Afterward, the coverslip was removed, 1% low-fusing-point drown gelose (85 μL) was mixed with separating lymphocytes uniformly. Approximately 85 μL of mixed liquor was obtained, dropped on the first agar lamination, covered with a coverslip, and then placed in a freezer at 4 °C for 5 min to 10 min. The prepared bilayer agar lamination was obtained, removed the coverslip, and placed in dissolving fluid at 4 °C for 60 min. infunded new-prepared alky-fluid in electrophoresis, and set the glass slip on crosser of electrophoresis for 40 min; voltage 18V, current flow 130-140 mA, for 30 minutes; each time for 3 minutes and 3 times together; dropped about 1 μg/m DAPI (4',6-diamidino-2-phenylindole) 20 μl on each agar lamination and covered coverslip.

"Comet" image semiquantitative analysis: the fluorescein-stained cellular nucleus was observed with fluorescence. One typical "comet" image includes the head and end, and the damage of different degrees shows five grades: 0, 1, 2, 3, 4, according to the ratio of the head and the end. Grade 0

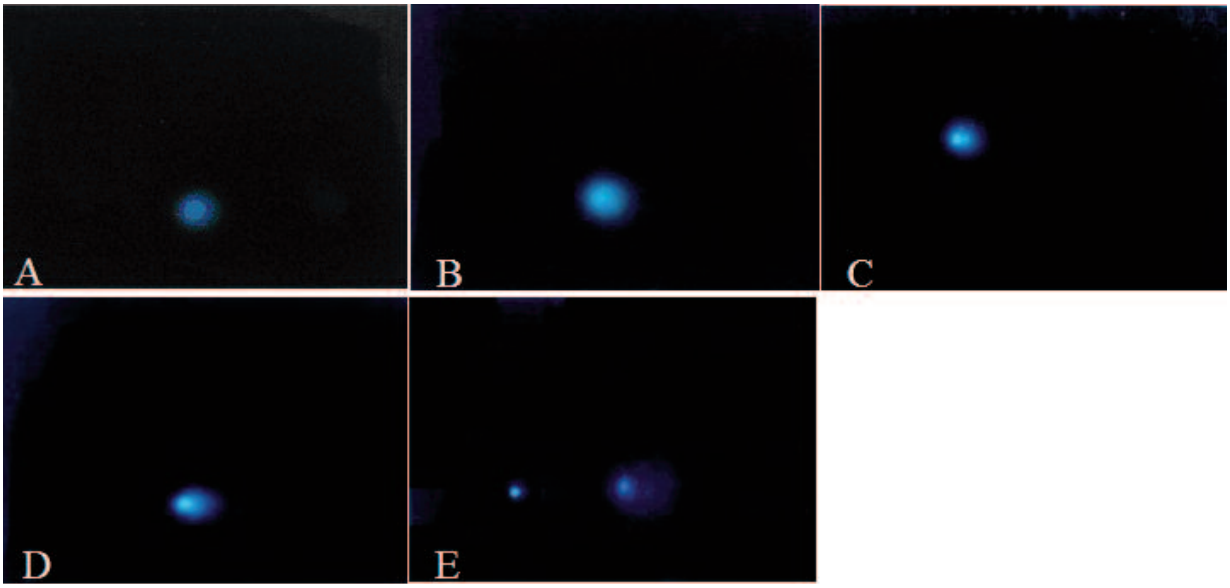

Figure 1. ***A,*** Grades 0 of DNA damage of the peripheral lymphocytes (×400). "Comet" image semiquantitative analysis: observe the fluorescein stained cellular nucleus with fluorescence. One typical "comet" image includes head and end, and damage of different degree shows five grades 0, 1, 2, 3, 4 according to the ratio of the head and the end. Grade 0 shows no damage with only its relucent disc but not the end. The end of "comet" would be longer and larger, and the head would be smaller with fluorescence intensity thinner as the degree of damage becoming severer. When experiment analysis, observe 100 "comet" cells in each example. The special unit AU (Arbitrary units) used in this article is a particular unit which is used to measure the degree of damage according to the size of head, the length of the end and the fluorescence intensity, and the whole level would be obtained by reforming and statistic different grades. ***B,*** Grades 1 of DNA damage of the peripheral lymphocytes (×400). ***C,*** Grades 2 of DNA damage of the peripheral lymphocytes (×400). ***D,*** Grades 3 of DNA damage of the peripheral lymphocytes (×400). ***E,*** Grades 4 of DNA damage of the peripheral lymphocytes (×400).

shows no damage, with only its relucent disc, but not the end. The end of the "comet" would be longer and larger, and the head would be smaller with thinner fluorescence intensity as the degree of damage becomes more severe. A total of 100 "comet" cells were observed in each example. The special unit AU (Arbitrary units) used in this article was a particular unit used to measure the degree of damage according to the size of the head, the length of the end, and the fluorescence intensity, in which the whole level would be obtained by reforming and statistic different grades (Figure 1).

Measurement of the MNF of PCE[23]

The femoral bone of mice was exposed and the two bone ends were snipped. Subsequently, with 1 mL syringe containing phosphate buffered saline (PBS) fluid was inserted in cavitas medullaris, the cavities were flushed, and the cell film was prepared. The film was then placed in a staining jar containing colonial spirit for 5 min to 8 min after air drying, and then dried in open air. The film was dyed for 10 min with newly prepared 10% Giemsa fluid, flushed with lotic water, and then measured with immersion objective after air drying. The number of micronucleus among the 1000 polychromato erythrocytes was counted. The field where cell appearance was accurate, disintegration was uniform, chromatosis was fair. PCE showed gray-blue, mature erythrocyte showed pink, typical micronuclei was singular, round, borderline slick and consistency, generally plum or amethyst in color, and diameter was 1/20-1/5 of red blood cells (RBC) usually (Figure 2).

Statistical Processing

The results were analyzed with mono-agent analysis of variance using SPSS11.5 statistical package (SPSS Inc., Chicago, IL, USA). Q-test was used to compare the results between two groups. A $p < 0.05$ was considered statistically significant.

Results

Liver Tissue MDA Content and SOD and GSH-PX Activity

The difference of liver tissue MDA content among the different groups was statistically significant ($F = 8.765$, $p < 0.01$). Among all the

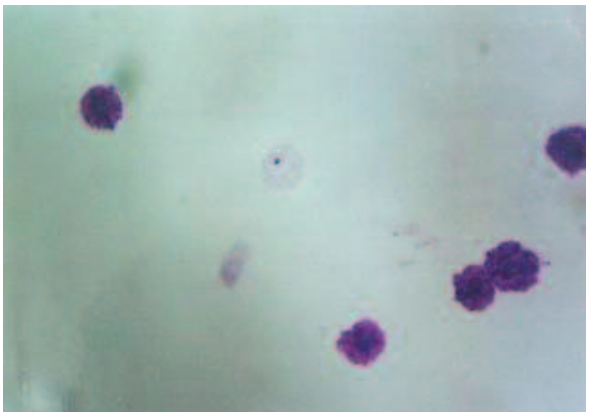

Figure 2. PCE showed gray-blue, mature erythrocyte showed pink, typical micronuclei was sing, round, borderline slick and regularity, prunosus or amethyst generally, and diameter was 1/20-1/5 of RBC usually.

groups, the control group had the lowest MDA, whereas the CTX group had highest. Compared with the control and sham-irradiated groups, MDA in the CTX group was increased significantly, with statistically significant difference ($p < 0.01$). Compared with the sham-irradiated group, the MDA in the LDR group had the tendency of decreasing, but was not significant ($p > 0.05$). Compared with the CTX group, the MDA content in the LDR+CTX group decreased significantly, and the difference was statistically significant ($p < 0.01$). The difference of the liver tissue activity of SOD among the different groups was statistically significant ($F = 8.511$, $p < 0.01$). Among all the groups, the CTX group had the lowest SOD activity, and the difference was statistically significant compared with the other groups. Compared with the sham-irradiated group, the SOD activity in the LDR group had tendency of increasing, but was not statistically significant ($p > 0.05$). Compared with the CTX group, SOD activity in the LDR+CTX group increased significantly, and the difference was statistically significant ($p < 0.01$). The difference of liver tissue activity of GSH-PX among the different groups was statistically significant ($F = 6.566$, $p < 0.01$). Among all the groups, the GSH-PX activity in the control group was the highest, and the difference was statistically significant compared with the other groups. Compared with the sham-irradiated group, the GSH-PX activity in LDR group had the tendency of increasing, but was not statistically significant ($p > 0.05$). Compared with the CTX group, the GSH-PX activity in the LDR+CTX group had the tendency of increasing, and was statistically significant ($p < 0.05$) (Table I).

Comparison of ALT and Protein Content in Plasma

The difference of ALT activity among the different groups was not significant ($F = 1.262$, $p > 0.05$). Among all the groups, ALT activity in the control group was the lowest. Compared with the control group, the ALT activity in the LDR group decreased; and that in the LDR+CTX group was lower than the CTX group. The difference of TP and ALB among different groups was significant ($F = 12.879$, $p < 0.01$; $F = 6.336$, $p < 0.01$). Among all the groups, the content of TP or ALB in the control group was the highest, and the difference was significant statistically compared with the other groups. The content of TP or ALB in the LDR group was higher than that of the control group, and the difference was significant ($p < 0.05$) (Table II).

Comparison of Peripheral Blood Lymphocyte DNA Damage

The difference of DNA damage among different groups was significant ($F = 6.383$, $p < 0.01$). Among all the groups, DNA damage in the blank control group was the lightest, whereas most serious in the CTX group. DNA damage in the cancer-

Table I. Liver tissue MDA content and activity of SOD and GSH-PX (x ± s).

Group	Number of Sample (n)	MDA (nmol/mgprot)	SOD (U/mgprot)	GSH-PX (activity unit)
Control group	9	1.15 ± 0.39	47.07 ± 6.09	240.39 ± 24.13
Sham-irradiation group	11	1.83 ± 0.41**	44.01 ± 5.19	206.07 ± 15.36**
LDR group	11	1.65 ± 0.43*	45.67 ± 4.95	219.69 ± 18.25*
CTX group	11	2.29 ± 0.52**	35.96 ± 4.85**	203.07 ± 12.74**
LDR+CTX group	11	1.77 ± 0.40**##	40.45 ± 3.73**#	219.71 ± 21.54*#

Note: compared with control group *$p < 0.05$, **$p < 0.01$; compared with CTX group #$p < 0.05$, ##$p < 0.01$

Table II. ALT and protein content in plasma (x ± s).

Group	Number of Sample (n)	ALT (U/L)	TP (g/L)	ALB (g/L)
Control group	9	58.00 ± 29.58	67.10 ± 3.44	31.92 ± 1.55
Sham-irradiation group	11	75.45 ± 19.83	58.79 ± 4.19**	28.94 ± 1.84**
LDR group	11	63.73 ± 7.39	62.14 ± 4.36**#	30.29 ± 1.54*#
CTX group	11	72.27 ± 17.39	57.15 ± 2.06**	29.62 ± 1.08**
LDR+CTX group	11	63.82 ± 22.35	59.47 ± 2.11**	30.54 ± 0.69*

Note: compared with control group $^{*}p < 0.05$, $^{**}p < 0.01$; compared with Sham-irradiation group $^{\#}p < 0.05$

bearing group was more serious compared with that in the control group ($p < 0.05$), whereas that in the LDR group was lighter compared with the control group, but was not significant ($p > 0.05$). DNA damage in the LDR+CTX group was lighter compared with that in the CTX group, and the difference was significant ($p < 0.05$) (Table III).

MNF of PCE

The difference of the MNF of PCE in each group was statistically significant ($F = 179.652$, $p < 0.01$). Among all the groups, MNF in the control group was the lowest, whereas the CTX group had the highest. Compared with the control and the sham-irradiated group, MNF in the CTX group increased significantly, and the difference was significant ($p < 0.01$). Compared with the sham-irradiated group, MNF in the LDR group had the tendency of decreasing, but was not significant ($p > 0.05$). The same result was observed in the LDR+CTX group compared with the CTX group (Table IV).

Discussion

In clinics, CTX can damage liver function, with the increase of alanine aminotransferase and bilirubin in the serum. The mechanism of liver function damage induced by drug may relate to oxidative stress[13]. Ni Xiuxiong et al[14] found function diminution in different damages of antioxidant defence system in liver tissue of S180 tumour-bearing mice with repeated injection of CTX into the abdominal cavity, whereas MDA content increased in the liver tissue. At the same time, pathological examinations found spot and mini-focal necrosis of liver cells. Both indicate that oxidative stress has a role in liver function damage induced by CTX. We obtained almost the same results with the above experiment. LDR before chemotherapy can significantly reduce the increase of MDA content caused by chemotherapy ($p < 0.01$), indicating that low dose pre-irradiation can lighten the damage of liver tissue caused by oxidative stress induced by high-dose CTX chemotherapy.

Continuous injections of CTX into the abdominal cavity may suppress the production of GSH, GSH-PX, SOD, and catalase[15,16]. Mice given pre-irradiation of 0.05 Gy to the total body before high dose irradiation had more activity of SOD and GSH-PX of liver cells than those only given high-dose irradiation. This result shows that low dose total body irradiation before high dose local irradiation may induce the antioxidant enzyme system of the body, elevate the activity of antioxidant enzyme, and has a protective role to the nor-

Table III. DNA damage of lymphocytes in each group (x ± s).

Group	Number of Sample (n)	Special Unit of DNA Damage
Control group	9	6.22 ± 2.73§
Sham-irradiation group	11	9.09 ± 2.66*§
LDR group	11	7.73 ± 3.29§
CTX group	11	12.91 ± 3.9#
LDR+CTX group	11	9.36 ± 2.98*△

Note: compared with control group $^{*}p < 0.05$, $^{\#}p < 0.01$; compared with which in control group $^{\triangle}p < 0.05$, $^{\S}p < 0.01$

Table IV. MNF of PCE in each group (x ± s).

Group	Number of Sample (n)	MNF (‰)
Control group	9	2.00 ± 0.71
Fake irradiation group	11	4.18 ± 1.83*
LDR group	11	2.55 ± 0.93
CTX group	11	25.18 ± 3.74#
LDR+CTX group	11	21.27 ± 4.17#

Note: compared with control group $^{*}p < 0.05$, $^{\#}p < 0.01$

mal tissues of an organism[17]. Our test shows that low dose irradiation before high dose chemotherapy can significantly increase the activity decline of GSH-PX and SOD caused by chemotherapy ($p < 0.05$). The outcomes indicate that low dose pre-irradiation may induce the antioxidant enzyme system, improves the activity decrease caused by high-dose CTX chemotherapy, and promotes the elimination of free radicals to decrease the side effects of chemotherapy. Our preliminary studies[18] showed an increase in the free radical of mice after CTX chemotherapy, the defence system of the SOD being destroyed, and a decrease in SOD activity of erythrocyte. Meanwhile, given the low-dose pre-irradiation and injections of CTX into the abdominal cavity after 6 hours, the SOD activity of erythrocyte rebounded and showed evident adaptive response.

Our study tested the ALT and albumin with automatic biochemical analyser, and did some preliminary research in this aspect. Our study explained from the lateral side that both tumor-bearing and high-dose chemotherapy could influence the liver function to a certain degree; but adding low dose irradiation may improve the liver function of protein synthesis.

A lot of chemical substances, including CTX, have some hepatotoxicity, but low dose pre-irradiation can alleviate this toxicity.

Injections of Fe^{3+}-NTA into the abdominal cavity could lead to activity peaks of liver enzymes after 12 hours, such as LDH, ALT, and AS. Low dose X-ray pre-irradiation to the whole body can inhibit the transient rise of these enzymes, and significantly enhance the SOD activity[8]. Our study showed that LDR has no effect on ALT activity of plasma, but may have protective effect on the protein synthesis function of the liver.

Karyocytes (such as lymphocytes and monocytes) in the peripheral blood disposite in whole blood circulation, including tumor cells, which are one kind of cells affected earliest and last longest by anticancer drugs[19]. Thus, we can comprehend the effect of low dose irradiation on the induction of DNA damage by CTX by investigating the DNA damage in peripheral lymphocytes.

Cheng et al[20] choose cancer-suffering patients who had been treated with CTX as viewing subjects, and detected DNA damage in the peripheral lymphocytes by CTX using single cell gel electrophoresis (SCGE). Their results showed that the DNA damage in the peripheral lymphocytes by CTX was more serious compared with the control group. In our experiment, we used S180 tumor-bearing mice as animal models, and examined DNA damage using SCGE. The results showed that low dose pre-irradiation before high dose chemotherapy may have protective effect on DNA damage, and the mechanism may be related to the repair of DNA damage and activity of antioxidase system induced by low dose radiation.

We only observed only light and little DNA damage, which may be attributed to the following reasons. (1) DNA damage in the peripheral blood lymphocytes was repaired at certain point. The repairing ability of ruptured DNA chains is different under different kinds of cells. For example, DNA damage of the peripheral blood lymphocyte preparation induced by ionizing radiation can be repaired within 3 h to 24 h[21]. The experiment cannot detect the DNA damage at different time points after chemotherapy. (2) CTX could cause DNA break and cross link, and the latter may be the main effect. DNA-protein cross link can block the movement of DNA fragments. (3) Cells damaged seriously by chemotherapy and apoptosis cells were soon cleaned up from blood circulation, so it was difficult to detect them.

Micronucleus is round or elliptic gonad nucleus which liberates in cytoplasm and separates from the primary nucleus, with a cellular diameter of 1/20 to 1/5 0f, and can be either a whole chromosome or chromosome break. The chromophile of the micronucleus is the same with the primary nucleus. Some micronucleus has the ability of duplicating DNA. Given that surrounding damage agents can break a chromosome, chromosome cannot enter the cell following the caryocinesia when cells enter the next caryocinesia; thus, chromosome elimination or fragmentation happens to form one or several micronucleus[22]. Various physical and chemical factors have certain effects on chromosomes, and affect its normal ability, destroy cellular DNA duplication and chromosome disintegration, and affect the function of spindle fibre to produce micronucleus. This damage is one type of damage in the chromosomal aberration at interval, and MNF can reflect the cell damaging degree by teratogenic factors and chromosome stability[4]. Compared with classic chromosome aberration analytic method, micronucleus experiment has no difference in sensibility, specificity, and accuracy, which has been proven by considerable internal and external check experiments[23]. PCE micronucleus experiment in mice marrow is widely applied to date. In the marrow, erythrocyte-series ancestral cells break up for several times, and then karyorrhexis occurs and be-

come mature erythrocytes. The essential component of HG-globulin in akaryocyte synthesis is produced earlier after karyorrhexis, and a devil of RNA in kytoplasm form PCE. As globulin synthesises completely, RNA breaks up completely and PCE gradually becomes mature NCE. When red cells undergo karyorrhexis at the last time, the micronucleus cannot be discharged from the kytoplasm through karyorrhexis, and remains in the cytoplasm and become MPCE[24].

Meng et al[24] believe that extensive co-toleration adaptation reaction is induced by low dose radiation between radiation and DNA cross linking agent, oxidative damage; whereas no cross resistance exists between radiation and aldyl agent, which could generate DNA alkylative damage. This experiment stated that low dose pre-irradiation did not show obvious protection against genetic toxicity by high-dose CTX chemotherapy, without synergistic action.

Zhou et al[25] observed that 0.075 Gy X-ray radiation had a depressant effect against dicaryon lymphocytes micronucleus induced by large dose X-ray radiation and MNNG. They considered that the appearance of adaptation reaction was induced by low dose radiation, which could stimulate some enzyme systems and strengthen the DNA repairing ability.

Conclusions

Our study showed that pre-chemotherapeutic LDR can induce the activities of anti-oxidative enzymes and promote the elimination of free radicals to alleviate the damaging effects of oxidative stress to hepatic tissue caused by high-dose CTX. At the same time, LDR had no effect on the ALT activity of plasma, but may have protective effect on the protein synthesis function of the liver. Furthermore, high-dose CTX chemotherapy can cause DNA damage of peripheral lymphocytes; whereas LDR before chemotherapy may have certain protective effect on DNA damage. CTX has potent mutagenic effect; however, LDR may not have protective effect against the genetic toxicity of CTX chemotherapy.

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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