·研究论文·

# THE EFFECTS OF LOW DOSE RADIATION (LDR) ON LYMPHOCYTES

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ABSTRACT LDR could stimulate lymphocyte transformation for adults, children and infants. The effect of LDR on lymphocytes in malnourished children was lower, but higher on lymphocytes in cord blood. The effect of LDR on $CD_4^+$ cells in adult persons was higher than that on $CD_8^+$ cells. NK cells were radioresistant. The stimulative effect of LDR on NK activeity in tumor patients was lower than that in normal individuals. For the mice with tumors, LDR could increase the ratio of $L_3T_4$ cells in blood, spleen and the number of cytotoxic T cells in the tumors. Extracellular fluid of the lymphocytes operated by LDR could also stimulate the lymphocyte transformation. The preliminary LDR could decrease the injuries to macromolecules membrane antigens and chromosomes in lymphocytes which were induced by high dose radiation. The LDR-induced protein might be found from mouse spleen cells, and this protein could increase immune function in human and animals.

KEYWORDS Low does radiation, Lymphocyte transformation, NK activity, Membrane antigen, DNA, Chromosome, LDR-induced protein
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## 1 Introduction

If factor in high dose has harmful effect on human and animals, but in low dose has beneficial effect, the latter effect is called hormesis. Radiation also has this characteristic, i.e. Low dose radiation (LDR) is experimentally proved healthful for human and animals.

LDR is defined as the radiation dose less than 0.2Gy for low LET(linear energy transfer) or less than 0.05Gy for high LET. In this case, when the dose rate is less than 0.05mGy/min, it is called low level radiation[1].

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Lymphocyte is a kind of important cells in organism, also a most radiosensitive kind, and it is easy to obtain from organism. Therefore lymphocytes are more suitable for study on radiation effect.

## 2 Stimulating effect

### 2.1 Stimulating effect of LDR on lymphocytes

Lymphocytes were irradiated by $^{226}$Ra with doses 2-50cGy at a dose rate 1.3cGy/h. After exposure, lymphocytes were cultured in 1640 medium with PHA, and $^{3}$H-TdR (for adults) or $^{14}$C-TdR (for children and cord blood) incorporation was used. (TdR is the precursor of DNA and according to our past experiments, both $^{3}$H and $^{14}$C labeling could reflect the DNA synthesis with the same regularity). Cells were harvested and their radioactivity was determined by liquid scintillation counter (Beckman, U.S.A.). The results were shown in table 1.

**Tab.1 Stimulating effect of LDR on human blood lymphocytes** (cpm%, $\overline{x} \pm s$)

| Doses /cGy | Adults ($n$=6) | >three years old children ($n$=9) | <three years old children ($n$=8) | Malnourished children ($n$=9) | Cord blood ($n$=10) |
|---|---|---|---|---|---|
| 0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 |
| 2 | 113.0±6.4 | 107.7±14.5 | 107.8±13.4 | 104.6±6.8 | 122.1±9.0$^{(1)}$ |
| 5 | 127.9±11.4$^{(1)}$ | 125.5±21.2$^{(2)}$ | 131.3±14.6$^{(2)}$ | 120.3±14.1$^{(1)}$ | 143.8±14.5$^{(2)}$ |
| 10 | 139.2±12.2$^{(2)}$ | 133.8±19.6$^{(2)}$ | 128.9±13.6$^{(2)}$ | 111.6±15.0$^{(1)}$ | 133.6±14.1$^{(2)}$ |
| 20 | 93.3±4.3 | 99.2±13.8 | 99.5±14.9 | 99.9±13.6 | 120.4±13.4$^{(1)}$ |
| 50 | 71.9±10.2$^{(1)}$ | 88.8±24.1 | 87.4±16.6$^{(1)}$ | 87.5±16.9 | 106.9±7.0 |

Compared with 0 cGy, $^{(1)}p < 0.05$, $^{(2)}p < 0.01$

The data in table 1 showed that in adult group the stimulating effects on lymphocyte transformation were obviously enhanced after exposure to 5cGy irradiation, and the highst stimulating effect was induced after 10cGy irradiation. In the child groups the effect was similar to that in the adult group. Cell-mediated immune function in malnourished children was originally lower than that in normal children ($p < 0.05$). After treatment with LDR, the stimulating effect on lymphocytes existed too in this group, but the enhancing degree was lower than that in normal children. When cord blood was irradiated with 2–20cGy, the immune functions were enhanced. It seemed that primary and young, the lymphocytes in cord blood was more radiosensitive.

### 2.2 Stimulating effect of LDR on lymphocyte subsets

$CD_4^+$, $CD_8^+$ and $CD_{19}^+$ (B) lymphocytes were isolated by the Panning direct method with monoclonal antibodies (McAb) and appraised with indirect immune fluorescence test respectively. The cell purity was more than 90% and the cell survival rate more than 97%. Three kinds of cells were irradiated by LDR respectively, and then they were cultured in medium with 10% AB serum and PHA ($CD_4^+$ and $CD_8^+$ cells) or LPS

($CD_{19}^+$ cells), and some monocytes were added into medium. $^3$H-TdR incorporation was determined with scintillation counter (Beckman). The results were showed in table 2.

**Tab.2 Stimulating effect of LDR on lymphocyte subsets** (cpm%, $\bar{x} \pm s$)

| Doses /cGy | $CD_4$ | | $CD_8$ | | $CD_{19}$ | |
|---|---|---|---|---|---|---|
| | Adults ($n$=6) | Cord blood ($n$=5) | Adults ($n$=6) | Cord blood ($n$=5) | Adults ($n$=6) | Cord blood ($n$=5) |
| 0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 |
| 2 | 168.4±25.3$^{(2)}$ | 113.7±13.4 | 143.3±30.1$^{(2)}$ | 125.0±15.4$^{(2)}$ | 159.0±23.2$^{(2)}$ | 119.1±8.3$^{(1)}$ |
| 5 | 176.6±31.5$^{(2)}$ | 146.9±21.4$^{(2)}$ | 169.6±25.4$^{(2)}$ | 145.4±15.3$^{(2)}$ | 175.4±15.6$^{(2)}$ | 157.9±8.6$^{(2)}$ |
| 10 | 179.3±31.6$^{(2)}$ | 148.3±20.3$^{(2)}$ | 163.7±24.3$^{(2)}$ | 142.7±14.8$^{(2)}$ | 182.5±10.3$^{(2)}$ | 156.0±23.6$^{(2)}$ |
| 20 | 145.4±19.7$^{(2)}$ | 134.0±18.4$^{(2)}$ | 126.7±17.2$^{(2)}$ | 117.9±12.6$^{(1)}$ | 139.6±21.8$^{(2)}$ | 123.0±19.3$^{(1)}$ |
| 50 | 38.4±2.0$^{(2)}$ | 116.3±16.0 | 38.6±9.2$^{(2)}$ | 78.9±8.5$^{(2)}$ b | 89.7±11.3 | 99.8±6.9 |

Compared with 0 cGy group, $^{(1)}p$ <0.05, $^{(2)}p$ <0.01

In adult group after 2–20 cGy irradiation, there was obvious stimulating effect ($p$ <0.01). Within the same dose range the stimulating effect on $CD_4^+$ cells was higher than that on $CD_8^+$ cells (For all doses, except 5cGy, the p values in comparison between any two data were less than 0.05). It is showed that the enhanced effect on $CD_4^+$ cells was higher than that on $CD_8^+$ cells, which reflected that the degree of enhancement of helping effect was higher than that of inhibiting effect. This mechanism might play an important role in immunity of organism.

LDR could also stimulate B lymphocyte transformation as $CD_4^+$ lymphocytes and could enhance the humoral immunity in organism.

In another experiment[2], LDR with 2–20cGy could enhance the helping effect of $CD_4^+$ cells on B cells and with 5–20cGy the inhibiting effect of $CD_8^+$ cells on B cells.

In the range of 2–20cGy, the effect of LDR on lymphocyte subsets in cord blood was very obvious, like in the adult group.

### 2.3 Stimulating effect of LDR on NK activity

NK cells isolated with $CD_{57}$ McAb and mononuclear cells isolated with the lymphocyte separation medium were used as effector cells respectively, while $K_{562}$ cells labeled by $^3$H-TdR as target cells. The NK cell activity or NK activity of lymphocytes were shown in table 3.

**Tab.3 Stimulating effect of LDR on NK activity** (cpm%, $\bar{x} \pm s$)

| Doses /cGy | NK cells | Lymphocytes | | |
|---|---|---|---|---|
| | Normal individuals ($n$=6) | Normal individuals ($n$=10) | Tumor patients ($n$=21) | Cord blood ($n$=6) |
| 0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 | 100.0±0.0 |
| 2 | | | | 132.6±8.9$^{(1)}$ |
| 5 | | | | 172.0±25.1$^{(1)}$ |
| 10 | 102.7±6.7 | 120.8±21.0$^{(1)}$ | 109.4±22.8 | 169.1±26.0$^{(1)}$ |
| 20 | | | | 158.4±33.4$^{(1)}$ |
| 50 | 128.5±8.7$^{(1)}$ | 140.0±21.4$^{(1)}$ | 120.5±9.8$^{(1)}$ | 115.2±22.7 |
| 80 | 134.2±12.3 | 108.4±17.5 | 98.5±10.6 | |

Compared with 0 cGy group, $^{(1)}p$ <0.01

When adult NK cells were irradiated by 50–80cGy, the NK activity enhanced significantly. After 50cGy irradiation, the growth of $CD_4^+$ and $CD_8^+$ cells were obviously suppressed, and the growth of B cells were also inhibited. It reflected that NK cells were radioresistant. When blood lymphocytes were irradiated with 10cGy, the NK activity increased significantly, but the NK activity of NK cells didn't increase until the irradiation dose reached to 50cGy. When given 80cGy irradiation, the blood lymphocytes showed no stimulating effect, but the NK cells showed the highest stimulating effect. It meant that the concepts of NK activity of NK cells and the NK activity of pan lymphocytes were different.

After 10 or 50cGy irradiation the NK activity of lymphocytes in normal individuals was enhanced, although the NK activity in the 50cGy group was increased more obviously. But in tumor patients only after 50cGy irradiation NK activity was enhanced significantly, and its intensity of enhancement was lower than that in normal individuals. It could be explained by that the NK activity in tumor patients was already suffered, and its potential function damaged.

After 2–20cGy irradiation, NK activity of the cord blood was obviously enhanced, and the increase intensity was higher than that in adults because of its higher radiosensitivity. In another experiment[3], there was synergic effect between $CD_4^+$ and NK cells. When $CD_4^+$ or NK cells were irradiated by 10cGy and 50cGy respectively, the total NK activity in these two kinds of co-cultured cells was higher than the sum of the activities of the two kinds of cells cultured separately. It showed that LDR was able to enhance the synergic action between $CD_4^+$ and NK cells. This phenomenon was not found between $CD_8^+$ or B cells and NK cells.

Our another experiment[4] showed that besides NK cells, $CD_4^+$, $CD_8^+$ and B cells could also kill tumor target cells. This result suggested once more that the concepts of NK activity of NK cells and total lymphocytes were different.

### 2.4 The stimulating effect of LDR on peripheral blood, spleen and tumor infiltrate lymphocytes (TIL) in mice with tumor

When Balb/c mice with tumors were exposed to whole body irradiation with doses 5cGy and 10cGy respectively, the $L_3T_4^+$ subsets (equivalent to $CD_4^+$ cells in human) in mouse peripheral blood and spleen increased and the $Lyt_2^+$ subsets (equivalent to $CD_8^+$ cells in human) decreased, and the ratio of $L_3T_4^+/Lyt_2^+$ in peripheral blood and spleen increased markedly. This result reflected that LDR could increase the number of T helper cells in peripheral blood and spleen, and the effect of LDR on immunity was enhanced. Nevertheless, the variation of $L_3T_4^+/Lyt_2^+$ ratio among TIL of irradiated mice was adverse to the above, and compared with control, the ratio decreased significantly[5]. It could be postulated that the ionizing radiation with low dose could activate cytotoxic

lymphocytes in the subset $Lyt_2^+$ to be accumulated in the tumor tissue, so that the proportion is increased, which might be significant in the tumor immunity.

**2.5 Stimulating effect of the extracellular fluid of lymphocytes exposed to LDR on the function of lymphocyte subsets**

The supernatant fluid of lymphocytes exposed to LDR could enhance the stimulating effect of lymphocyte subsets activated by PHA. The result showed that DNA synthesis in $CD_4^+$ cells is enhanced in a dose range from 0.5 to 8cGy and that in $CD_8^+$ cells from 1to 4cGy, and in the same dose range the stimulating effect of the supernatant fluid on $CD_4^+$ cells was higher than that on $CD_8^+$ cells[6], which showed that the stimulating effect of LDR on $CD_4^+$ cells was higher than that on $CD_8^+$ cells. These results also extended the experimental data about that LDR could enhance the helping effect.

## 3 LDR-caused adaptive response of lymphocytes to challenge irradiation

Lymphocytes irradiated by LDR ($D_1$) could decrease the damage to cells induced by a following challenge dose ($D_2$).

**3.1 Synthesis of biological macromolecules**

Exposure of lymphocytes to preliminary 4.8cGy radiation could decrease the damage to DNA, RNA and protein synthesis in the cells caused by the challenge doses (0.5 to 8Gy) of radiation[7,8].

**3.2 Membrane antigens**

Using the McAb of $CD_4$, $CD_8$ or $CD_{20}$ (B) labeled by $^{125}I$, the radiation-induced damage to the membrane antigens and their adaptive response were detected by radioactive determination. The results showed that high dose radiation could do damage to membrane antigens in lymphocytes. 4.8cGy preliminary irradiation could induce adaptive response to the following challenge irradiation (0.5 to 4Gy). The greatest response occurred with the dose of 0.5Gy (compared with control, $p < 0.05$).

In another experiment[9], the effects of $\gamma$ rays irradiation on $CD_3$, $CD_4$ and $CD_8$ expressions in human lymphocytes which were irradiated with 2, 4, and 8Gy $\gamma$ rays respectively or with 2, 4 and 8Gy ($D_2$) $\gamma$ rays respectively 4h after 10cGy irradiation ($D_1$) were investigated by flow cytometry with immunofluroescence technique. The results showed that the $CD_3$, $CD_4$ and $CD_8$ expressions in lymphocytes in highly irradiated groups decreased significantly as the dose increased, while the previous exposure of $CD_3$, $CD_4$ and $CD_8$ to 10cGy irradiation could reduce this influence ($p < 0.05$).

**3.3 DNA strand break**

Fluorometric analysis of DNA unwinding (FADU) was used to reflect the adaptive response of lymphocytes. The results[10] showed that for the damage to DNA strand break, $\gamma$ rays with a dose of 0.5–4cGy could induce adaptive response, especially in the case of 2.0cGy ($D_1$) and 15Gy ($D_2$). 0.5mmol/L 3-AB (3-Amino benzoylamide, the

PADPRT inhibitor) could inhibit the adaptive response induced by γ rays irradiation, which showed that the PADPRT might play an important role in the mechanism of adaptive response. Repair (at 37°C, for 15–60min) of the DNA strand break caused by irradiation with 15Gy could be promoted by a 2cGy preliminary irradiation.

### 3.4 Chromosome aberration

Fluorescence in site hybridization (FISH) with the whole chromosome 4 painting probe was used to analyze radiation-induced chromosome aberrations. When lymphocytes were exposed to a dose from 0.5 to 2.0Gy ($D_2$), chromosome aberrations acentrics, dicentrics, ring and translocation showed their obvious dependence on the doses. After 7.5cGy irradiation ($D_1$), acentrics and translocation induced by $D_2$ decreased significantly, which indicated that the ability of cells to repair chromosome aberration was raised by adaptive response (table 4).

**Tab.4 Chromosome aberrations induced by 7.5cGy preliminary irradiation dose followed by a $D_2$ challenging irradiation dose** (number of aberration cells (%))

| $D_2$/Gy | Group | Cells observed | Acentrics | Dicentrics | Ring | Translocation |
|---|---|---|---|---|---|---|
| 0.0 | $D_2$ | 3947 | 3(0.08) | 0(0.00) | 0(0.00) | 4(0.10) |
| | $D_1+D_2$ | 3946 | 3(0.08) | 0(0.00) | 0(0.00) | 4(0.10) |
| 0.5 | $D_2$ | 3571 | 14(0.39) | 12(0.34) | 1(0.03) | 20(0.56) |
| | $D_1+D_2$ | 4000 | 10(0.25) | 8(0.20) | 1(0.03) | 12(0.30) |
| 1.0 | $D_2$ | 1924 | 20(1.04) | 14(0.73) | 2(0.11) | 28(1.46) |
| | $D_1+D_2$ | 2539 | 16(0.63) | 11(0.43) | 1(0.04) | 17(0.67)$^{(2)}$ |
| 2.0 | $D_2$ | 1040 | 30(2.88) | 28(2.69) | 4(0.38) | 50(4.80) |
| | $D_1+D_2$ | 1372 | 20(1.46)$^{(1)}$ | 23(1.68) | 4(0.29) | 36(2.62)$^{(2)}$ |

Compared with $D_2$ group, $^{(1)}p<0.05$, $^{(2)}p<0.01$

The formula was expressed as follows:

$$\text{Total aberration declining rate}=\left(1-\frac{\text{total aberration rate of } (D_1+D_2) \text{ group}}{\text{total aberration rate of } D_2 \text{ group}}\right)\times 100\%$$

The declining rate induced by various challenging doses was 41% for 0.5Gy group, 46% for 1.0Gy group, 44% for 2.0Gy group, respectively, and the best repair ability was found in the 1.0Gy group.

## 4 LDR-induced protein expression in lymphocytes

The spleen cells of mice were exposed to LDR, and then the cells were lysed with sonication and ultracentrifugalized at low temperature. The solution was filtered with sephadex -G100 gel, and the new emerging protein was determined with SDS polyacrylamide gel electrophoresis and quantified with lowry's method. The LDR-induced protein might be found from the mouse spleen cells which had been exposed to 5–15cGy irradiation for 2–16h.

The protein had biological activity and might stimulate the transformation of spleen cells in vitro. The biological effects increase with the quantity of quantity of the quantity of LDR-induced protein within certain limits. Some factors such as high dose of ionizing

radiation, UV radiation, heat, acid and alkali might cause lymphocyte impairments. The LDR-induced protein had obvious protective effects on these impaired lymphocytes. This protein had also stimulating effects on the transformation of T and B lymphocytes in peripheral blood of normal individuals and patients with eye disease such as glaucoma, cataract, uveitis, which reflected that the LDR- induced protein could enhance immune function in human[11].

In conclusion , in this paper, LDR showed its multifold effects on lymphocytes. LDR could stimulate the transformation of lymphocytes and their subsets. Especially, the stimulating effect on T helper cells was higher than that on T suppressor cells, which showed an important role of LDR in enhancing immune function in organism. Lymphocytes in cord blood had higher radiosensitivity than that in adult blood. NK cells were radioresistant, which meant that it took higher dose to enhance the NK activity. Research also showed the adaptive response induced by LDR of lymphocytes which were operated with a following challenge dose radiation, i.e. preliminary LDR could decrease the injury to macromolecules, membrane antigens and chromosomes caused by high dose radiation. When the spleen cells of mice were exposed to LDR, the new emerging protein could be found. This protein had obvious protective effect on impaired lymphocytes and stimulating effect on lymphocytes of normal individuals, patients and animals.

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# 低剂量辐射(LDR)对淋巴细胞的效应

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**摘要** LDR 能刺激成人、儿童和婴幼儿的淋巴细胞转化，对营养不良儿童的刺激效应较低，对脐血则刺激效应较明显。LDR 对成人 $CD_4$ 细胞的刺激效应强于 $CD_8$ 细胞，而以同样的刺激效应，NK 细胞则需要较大剂量。对淋巴细胞 NK 活性也有同样规律。肿瘤病人的 NK 活性的刺激效应低于正常人。LDR 增加了小鼠的血和脾内的 T 辅助/诱导细胞的比例和肿瘤内 T 细胞毒性细胞。低剂量照射的淋巴细胞外液也能刺激淋巴细胞转化。预先 LDR 可以减轻随后大剂量照射引起的生物大分子、膜抗原和染色体的损伤。LDR 可诱导鼠脾淋巴细胞产生新蛋白，它能增强人和动物的免疫功能。

**关键词** LDR，淋巴细胞转化，NK 活性，膜抗原，DNA，染色体，LDR 诱导蛋白
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