Low dose effects on cultured mammalian cells ···

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Abstract The low dose effects induced by carbon ions on Chinese hamster V79 cells and murine melanoma B16 cells were investigated in this paper. Both cell lines were divided into four groups for irradiation: (1) control, (2) 0.02 Gy or 0.05 Gy($D1$), (3) 1 Gy($D2$), (4) $D1$+$D2$. The survivors and micronuclei were studied as biological endpoints. The results of group (1) and group (2) showed that there were no obvious differences on micronucleus frequency but there were significant increases when irradiation dose was 0.02Gy on colony formation efficiency. Although low dose ion irradiation could not contribute to DNA damages, it could enhance the colony formation efficiency. In the study of group (3) and (4), when the ion dose was 0.02 Gy, there were evident increases on surviving fraction and decreases on micronucleus frequency, but there were no statistical changes on these endpoints when the ion dose was 0.05Gy. This meant that high LET radiation could induce the adaptive response of cultured cells, furthermore, in the range of inducing ion dose , low dose irradiation was more profitable than high dose one.

Keywords Low dose effect, Carbon ions, Mammalian cell, Adaptive response, Surviving fraction, Micronucleus frequency, LET(linear energy transfer)

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1 INTRODUCTION

It is well known that ionizing radiation can induce many deleterious effects in cells, for instance, chromatid aberrations, mutations and killing of cells. But in 1980s, it was found that low level of ionizing radiation could stimulate the growth, proliferation of animals, plants and microbes (Luckey, 1980)[1]. Human lymphocytes treated with a low dose of ionizing irradiation prior to a high dose exposure could have fewer chromatid aberrations than expected (Olivieri, 1984)[2], this phenomenon had been termed as adaptive response to ionizing radiation, and has been observed by a lot of scientists. Most of their work focused on low LET radiations, such as X, γ and β rays, as for high LET radiations few investigations have been done. It was reported that pre-exposure of lymphocytes to X-rays could reduce the chromosomal damages induced by α particles (Wolff, 1991)[3]. In 1992, Brooks[4] injected radioisotope ^{239}Pu, which irradiates high LET α particles, into Chinese hamsters, at 30th and 410th day, they observed the bone marrow cells after 2 Gy whole body γ-ray irradiation. The results showed that there was remarkable decrease on

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chromosomal exchanges at 30th day, but not at 410th day. Recently, it was reported that pre-exposure of mouse testis to a low dose of $^{16}O^{8+}$ beams rendered the organ of mouse more resistant to subsequent high dose irradiation (Zhang, 1998)[5]. This suggested that high LET heavy ions could induce the adaptive response of mouse. These results made it very interesting to study the effects of low dose of heavy ions further.

In this study, the survivors and micronuclei of cultured Chinese hamster V79 cells and murine melanoma B16 cells after irradiated with low doses of $^{12}C^{6+}$ beams were investigated.

2 MATERIALS AND METHOD

2.1 Cells and cell culture

The cell lines for test were Chinese hamster V79 cells and murine melanoma B16 cells (purchased from Institute of Cancer Research, Beijing). Cells were subcultured every 3d in RMPI-1640 medium (Gibco, Europe) with 10% newborn calf serum. The cultures were maintained at 37°C in a humidified incubator containing 5% CO_2. Before irradiation, cells were plated in ϕ35 mm Petri-dishes at a concentration of 5×10^4 cells/mL and cultured for 24 h.

2.2 Irradiation

According to the UNSCEAR report in 1986, low level of ionizing radiation were these doses which were not higher than 0.2 Gy of low LET radiation or 0.05 Gy of high LET radiation. So in this study, 0.02 and 0.05 Gy ($D1$) were chosen as low doses at a dose rate of 0.08 Gy/min approximately, 1 Gy ($D2$) as subsequent high dose at a dose rate of 2 Gy/min. Four groups for each type of irradiation were divided as (1) control, (2) $D1$, (3) $D2$, (4) $D1+D2$, in group(4), before irradiated with high dose of $D2$, cells were incubated for 4 h at 37°C.

The irradiation was performed at the Heavy Ion Research Facility in Lan Zhou (HIRFL) in dishes attached to 2μm thin Mylar foil. Cells were exposed to $^{12}C^{6+}$ beams with energy of 50 MeV/u.

2.3 Survival assay

After irradiation, cells were trypsinized and plated in ϕ60mm plates at 100 cells per plate to assay the colony forming ability. After incubation for 8 d, cells were stained with Giemsa dye, colonies that had more than 50 cells were scored as survivors. The colony formation efficiency (CFE) was determined by following equation:

$$CFE(\%) = (N_x/N)\times 100\%$$

here N_x was the number of colonies, N was the number of cells plated.

The survival fraction (SF) was calculated by following equation:

$$SF(\%) = (S_x/S_0)\times\ 100\%,$$

S_x was colony formation efficiency of irradiated cells, S_0 was colony formation efficiency of control.

2.4Micronucleus assay

At the same time of survival assay, trypsinized cells were plated in ϕ35 mm dishes at appropriate dilutions and cultured for 24 h, the proportion of cells with micronuclei in total cell population was determined using a fluorescence microscope.

3 RESULTS AND DISCUSSION 4

In the study of group (1) and (2), the differences of micronucleus frequency were not obvious, this was under our expectance, since the low dose was too low to contribute to DNA damages. But there were significant increases on colony formation efficiency when the dose was 0.02 Gy, as shown in Table 1.

It was reported (Smith, 1992)[6] that exposure of human fibroblasts to low dose of γ-rays caused an increase on colony formation efficiency, but there were no such phenomena in human fibroblast mutants which were deficient in DNA repair, so it was deduced that low dose could not contribute to DNA damage, but could induce enhanced colony formation efficiency with a new type of DNA repair mechanism.

The adaptive response of cultured cells was studied in group (3) and (4). The results of surviving fraction of both cell lines were shown in Table 2. The surviving fractions of cells pre-exposed to 0.02 Gy were significant higher than the results of cells which were only exposed to 1Gy. No significant differences were found between cells pre-exposed to 0.05 Gy and exposed to 1 Gy alone.

Table 1 The colony formation efficiency (%) of V79 and B16 cells in group (1) and (2)

Cell	Dose/Gy		
line	control	0.02	0.05
V79	16±1.7	27±1.9$^{(1)}$	19±2.1$^{(2)}$
B16	25±1.8	42±2.4$^{(1)}$	25±2.0$^{(2)}$

Data shown in this table were mean volumes of 4 independent culture dishes. $^{(1)}$Significant height, p <0.05, compared with the results of control, $^{(2)}$No significant difference, p >0.05, compared with the results of control

Table 2 The surviving fraction (%) of V79 and B16 cells in group (3) and (4)

Cell	Dose/Gy		
line	1	(0.02+1)	(0.05+1)
V79	47±9.4	96±8.7$^{(1)}$	56±13.0$^{(2)}$
B16	56±5.2	78±5.2$^{(1)}$	45±10.0$^{(2)}$

Data shown in this table were mean volumes of 4 independent culture dishes. $^{(1)}$Significant height, p <0.05, compared with the results of 1 Gy. $^{(2)}$No significant difference, p >0.05, compared with the results of 1 Gy

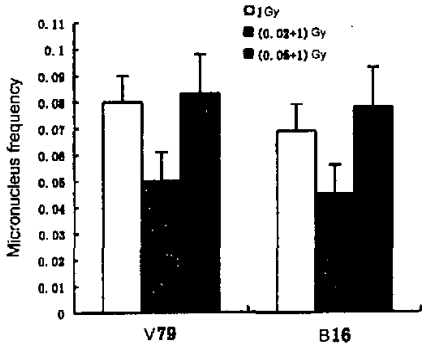

Fig.1 Micronucleus frequency of V79 cells and B16 cells irradiated by carbon ions

The results of micronucleus frequency of group (3) and (4) were shown in Fig.1. One thousand cells were scored for every point. The micronucleus frequency of cells pre-exposed to 0.02 Gy decreased 38% (p <0.05) and 35%(p <0.05) in V79 and B16 cells, respectively. Meanwhile, the micronucleus frequency of cells pre-exposed to 0.05 Gy increased 4%(p >0.05) and 11%(p >0.05) in V79 and B16 cells, respectively. Although these increases were not significant, pre-exposure cells to 0.05 Gy had shown more serious injuries.

In the study of Brooks, at 30th day after injection of ^{239}Pu, the absorbed dose $D1$ equivalent of Chinese hamster was 0.1 Sv, there was adaptive response on chromosomal exchanges. At 410 th day, the absorbed dose $D1$ equivalent was 1.44 Sv, no adaptive response was found. In our experiment, samples were irradiated with the plateau of carbon ions, in this case, the RBE of heavy ions was about 2, so 0.02 Gy and 0.05 Gy were equivalent to 0.04 Sv and 0.1 Sv, respectively. Our results showed that 0.02 Gy could induce survival and micronucleus adaptive response while 0.05 Gy could not. This agreed with the results of Brooks that in the range of inducing ion dose, low dose irradiation was more profitable to induce adaptive response than high dose one.

On the other hand, although whole body irradiation of 0.05 Gy oxygen ions could induce adaptive response of mice in the experiment of Zhang's[5], 0.05 Gy of carbon ions could not induce adaptive response of cultured cells, this might due to the different condition between vivo and vitro, or kind of ions and so on.

From the results of all above, we conclude that low level of high LET irradiation such as 0.02 Gy carbon ions could enhance the colony formation efficiency and induce survival and micronucleus adaptive response of cultured cells.

References

1 Luckey T D. Hormesis with ionizing radiation, CRC Press, 1980, 1~122
2 Olivieri G, Bodycote J, Wolff S. Science, 1984, **223**(5):594~597
3 Wolff S, Jostes R M, Cross F T *et al.* Mutat Res, 1991, **250**(3):299~306
4 Brooks A L, Mcdonald K E, Kimsey B B *et al.* Low dose irradiation and biological defense mechanisms, Elsevier Science Publishers, 1992, 391~ 322
5 Zhang H, Zheng R L, Wei Z Q *et al.* Int J Radiat Biol, 1998, **73**(2):163~167
6 Lui S Z. Hormesis with low level of ionizing radiation (in Chinese), Beijing: Science Press, 1996, 181