Chromatin and DNA chain fragmentation studies on apoptosis in immune cells induced by ^{235}U and radioprotection of IL-2 or IL-6*

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Abstract The apoptosis in human acute lymphoblastic leukemia cell line Molt-4 cell and macrophage cell line Ana-1 cell are studied after internal irradiation with enriched uranium ^{235}U. The cumulative absorption dose of ^{235}U in cultural cells through different periods are estimated. The fluorescence microscopic observations indicate that Molt-4 and Ana-1 immune cells internally irradiated by ^{235}U displayed significant chromatin fragmentation and marked pyknosis in immune cells nuclei, as well as DNA chain fragmentation and apoptotic bodies formation. It should be noted that DNA chain fragmentation induced by ^{235}U may be inhibited statistically by IL-2 (interleukin-2) or IL-6 treatment.

Keywords Chromatin, DNA chain fragmentation, Apoptosis, Molt-4 cell, Ana-1 cell, Enriched uranium, IL-2, IL-6

1 Introduction

In recent years, nuclear power plants have been built, therefore, the utilization of nuclear fuel enriched uranium ^{235}U has been got a rapid development.[1] The environmental pollution and damage to human beings by nuclear fuel and its fission products released from nuclear tests and plants are more and more concerned by the public.[2] With an increase in production of enriched uranium ^{235}U, observations of its effect on environment and on human beings become a significant task.[3] In this respect, people are paying close attention to study the immune toxicity in organisms induced by enriched uranium ^{235}U. It should be noted that study on apoptosis in immune cells induced by internal irradiated radionuclides become an important work[4], but no such studies were reported yet. A study on apoptosis in immune cells induced by different radioactivities of ^{235}U is presented in this paper.

2 Experimental methods and results

2.1 Cell culture

A human acute lymphoblastic leukemia cell line Molt-4 cell and a macrophage cell line Ana-1 cell stemming from Shanghai Immunological Institute were maintained in RPMI 1640 (GIBCO), supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, 2 mmol/L L-glutamine as well as 5×10^{-5} mol/L 2-mercaptoethanol, which was known as the complete RPMI 1640 medium. Cells were kept in an atmosphere containing 0.05 volume fraction CO_2 at 37°C and used in exponential growth. Then Molt-4 and Ana-1 immune cells, internally irradiated with enriched uranium ^{235}U, were washed three times with Ca^{++} and Mg^{++} free Hanks solution, and adjusted to a concentration of 2×10^6 cells/ml in complete RPMI 1640 medium.

2.2 Absorption dose estimation of enriched uranium ^{235}U in cultural cells

$^{235}UO_2F_2$ with radioactive and chemical purity was used in this work. At first, 1 ml of enriched uranium ^{235}U solution with radioactivities 128.4 Bq/ml in complete RPMI 1640 medium was added into Molt-4 and Ana-1 cell suspensions at 2×10^6 cells/ml. Then, the solution was incubated in a 5% CO_2 atmosphere at 37°C through 3, 6, 9, 12, 24 and 48 h. The results are listed in Table 1. The cumulative radiation absorption dose (D, mGy) is calculated according to the following formula.[5]

$$D = AEt/m$$

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where A is radioactivities, Bq; E average energy of α particles, MeV; t time of irradiation, h; m mass of the cells.

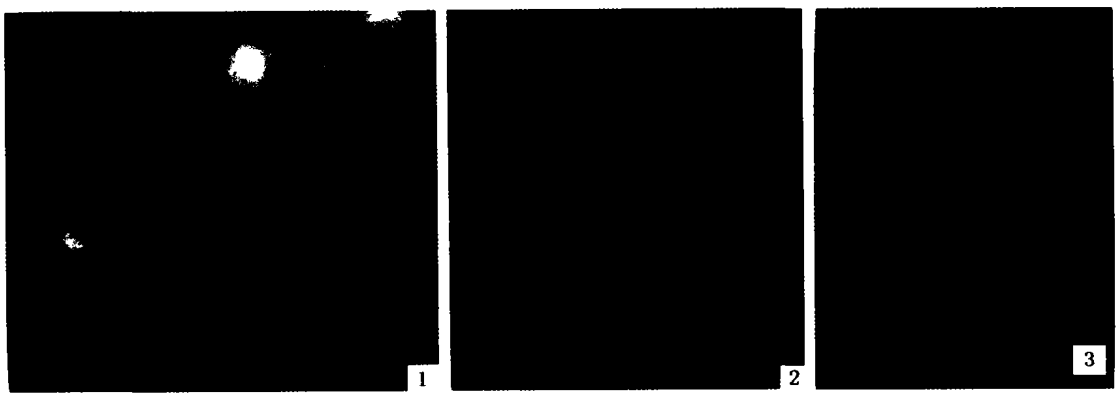
Table 1 Estimated cumulative absorption dose of internal irradiation with ^{235}U in Molt-4 and Ana-1 cells through different periods

Internal irradiation periods/h	3	6	9	12	24	48
Cumulative absorption dose/mGy	0.49	0.98	1.47	1.96	2.91	5.82

2.3 Fluorescence microscopic observation

Experimental Molt-4 and Ana-1 immune cells were suspended in complete RPMI 1640 medium to form suspension at 2×10^6 cells/ml. The cell suspensions of 1 ml were added to 24-well microtitration plate. Then 1 ml of enriched uranium ^{235}U with radioactivities 128.4 Bq/ml also in complete RPMI 1640 medium was added to each experimental well. But to control wells was added 1 ml RPMI 1640 medium only. The microtitration plates were then incubated in a 5% CO_2 at 37°C through 3, 6, 9, 12, 24 and 48 h. After internal irradiation with ^{235}U for different periods, Molt-4 and Ana-1 cells were harvested and washed 5 times with Hanks solution to take away the free radioactivities of ^{235}U. After a while, Molt-4 and Ana-1 immune cells were suspended into hypotonic fluorescent solution, which consists of 50 mg/ml pyridine iodide and 0.1% sodium acetate as well as 0.1% triton X-100[6], in order to examine the morphology of Molt-4 and Ana-1 immune cell nuclei.

Fig.1 shows that heterogeneous nuclear chromatin appears in the Molt-4 cells, at the same time the Ana-1 cells also show heterogeneous nuclear chromatin. While Fig.2 gives marked chromatin fragmentation and pyknosis in PI-stained Molt-4 cell nuclei after 24 h incubation with 64.2 Bq/ml ^{235}U, Fig.3 shows marked fragmentation in chromatin of Ana-1 immune cells.



Figs.1-3 Fluorescence microscopic appearance of PI-stained nuclei of the control Molt-4 cell after 12 h incubation in RPMI 1640 medium alone (1), of PI-stained Molt-4 cell nuclei after 24 h internal irradiation with 64.2 Bq/ml ^{235}U (2), and of PI-stained Ana-1 cell nuclei after 12 h internal irradiation with 64.2 Bq/ml ^{235}U (3) ×1250

2.4 DNA chain fragmentation induced by ^{235}U and the radioprotection of IL-2 and IL-6

Molt-4 and Ana-1 immune cells were harvested, washed with Hanks solution and centrifuged. Thereafter, the suspension of cells was adjusted to a concentration of 2×10^6 cells/ml in complete RPMI 1640 medium. Then 37 kBq ^3H-TdR[7] was added to each 2×10^6 cells/ml immune cells and was incubated in a 5% CO_2 atmosphere at 37°C through 6 h. The labelled Molt-4 or Ana-1 cells were harvested again and

washed with Hanks solution in order to remove the free ^{3}H-TdR. 1 ml of Molt-4 or Ana-1 cells suspension in complete RPMI 1640 medium of 2×10^{6} cells/ml was added to each well of 24-well microtitration plate.[8] Then 1 ml of ^{235}U with 128.4 Bq/ml in complete RPMI 1640 medium was added to each experimental well containing 1×10^{3} U/ml of rIL-2 or 1.9×10^{3} U/ml of rIL-6 in order to detect the radioprotective effect. To each control well, 1 ml RPMI 1640 medium was added only. The microtitration plates were then incubated in humidified atmosphere of 5% CO_2 incubator at 37°C through 3, 6, 9, 12, 24 and 48 h. After each interval culture, the cells of each sample were gathered by multicanal cell collector apparatus onto a No.49 glass fibre filter, dried at 50°C, dropped into 5 ml scintillation cocktail with 0.4% PPO and 0.04% POPOP in xylene. Their radioactivities were determined with a Beckman LS 6800 liquid scintillation counter. Experimental results are expressed as:

Fraction of DNA *chain fragmentation, %= (control* cpm - *experimental* cpm*)/ control* cpm

It can be seen from Table 2 that the fraction of DNA chain fragmentation either in Molt-4 or in Ana-1 immune cells increases progressively, while the interval of ^{235}U internal irradiation prolongs gradually.

Table 2 The radioprotection of IL-2 and IL-6 on DNA chain fragmentation of Molt-4 and Ana-1 cells induced by ^{235}U

Time/h	Control	IL-2	IL-6
Percent of Molt-4 cell DNA chain fragmentation			
3	37±4	5±0.5**	9±0.4**
6	41±6	17±1**	17±1**
9	54±3	30±3**	23±1**
12	67±8	39±5**	35±4**
24	75±7	44±6**	39±4**
48	76±8	48±4**	53±3**
Percent of Ana-1 cell DNA chain fragmentation			
3	19±2	16±1	8±0.9**
6	26±2	19±1	7±1**
9	39±3	20±2**	8±0.6**
12	56±8	21±2**	14±1**
24	62±2	35±4**	32±4**
48	71±8	52±4**	33±2**

**$P < 0.01$

As shown in Table 2, the addition of rIL-2 as well as rIL-6 to the cultured Molt-4 or Ana-1 immune cells markedly decreases the DNA chain fragmentation. It should be pointed out that exogenous IL-2 or IL-6 at different experimental intervals shows statistically significant radioprotective effect.

3 Discussion

Previous works were directed towards external radiation induced apoptosis in cells.[9] Now by using fluorescence microscopy, it can be found that Molt-4 or Ana-1 cells internally irradiated by ^{235}U display obvious chromatin fragmentation and marked pyknosis in immune cells nuclei, as well as DNA chain fragmentation and apoptotic bodies formation. These characteristics suggest that the immune cells could be induced by internal irradiation of ^{235}U to undergo apoptosis.

Our experimental results indicate that apoptotic cell formation in Molt-4 or Ana-1 immune cells induced by enriched uranium ^{235}U depends on the ^{235}U-treated time as well as accumulated absorption dose. As shown above the percent of DNA chain fragmentation increases with the prolongation of internal irradiation time by ^{235}U. It should be pointed out that DNA chain fragmentation induced by ^{235}U can be inhibited statistically by rIL-2 or rIL-6 treatment. Therefore, both IL-2 and IL-6 have radioprotective effect on Molt-4 and Ana-1 immune cells irradiated by internal exposure of enriched uranium ^{235}U.

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