RADIOIMMUNOTOXICOLOGICAL EFFECT OF ENRICHED URANIUM ON CENTRAL AND PERIPHERAL IMMUNE CELLS AND THE PROTECTIVE ACTION OF IL—1 AND IL—2*

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ABSTRACT

Accumulation of enriched $^{235}U-UO_2F_2$ in the body had injurious effects on the immune function of central and peripheral immune cells. After an intravenous injection of $^{235}U-UO_2F_2$, the spontaneous $^{3}H-TdR$ incorporation in thymocytes and bone marrow cells decreased, with the thymocytes damaged more markedly. The proliferation ability of spleen T and B lymphocytes were both inhibited, with B lymphocytes inhibited more severely. In spleen B lymphocytes the IL-1 production and IL-2 consumption were diminished. The inhibition of spleen B lymphocyte proliferation by $^{235}U-UO_2F_2$ was partially restored by adding exogenous IL-1 or IL-2 to the cultured lymphocytes obtained from ^{235}U injected mice.

Keywords Enriched uranium, Radioimmunotoxicological effect, Interleukin-1, Interleukin-2, Immune cells, Protective action

1 INTRODUCTION

Enriched ^{235}U is one of the principal fuels of nuclear power stations. Since new nuclear power stations are increasing rapidly, the production and consume of enriched ^{235}U expand year by year. Consequently, the study of its harming effects on animal and human bodies become and urgent task. Among the various harming effects, the radioimmunotoxicity deserves particular attention.

In the environment of nuclear fuel production, either soluble or insoluble uranium may contaminate the body by different ways[1,2]. The harming effects of enriched uranium, especially its immunosuppression effect, is closely related to its retention characteristics and is still a problem not yet extensively studied. We therefore carried out a study on the radioimmunotoxic effect of enriched uranium on central andperipheral immune cells and the protective action of exogenous Interleukin-1 (IL-1) and Interleukin-2 (IL-2).

2 MATERIALS AND METHODS

2.1 Reagents

Enriched uranyl fluoride with 18.9% abundance of ^{235}U was used in this study.

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Sexually mature BALB/c mice, 6—8 weeks old and weighing 19.2 ± 2 g, were divided into control and experiment groups. Mice of the experimental groups were each injected intravenously with 20 mg/kg of $^{235}U-UO_2F_2$.

Mitogenic PHA was from Shanghai Medical Test Institute, LPS and ConA were from Sigma, IL-1 was from Hoffman La Roche, IL-2 was from Shanghai Institute of Biochemistry, and ^3H-TdR with a specific activity of 37 MBq/mmol was from Shanghai Institute of Nuclear Research.

The cell culture medium, known as complete RPMI 1640, was composed of RPMI 1640 (J R Scientific), supplemented with 20mmol/l HEPES, 2 mmol/l L-glutamine, 3.6 g/l glucose, 2.0 g/l sodium bicarbonate, 5×10^{-5} mol/l 2-mercaptoethanol, 100 μg/ml streptomycin, 100 u/ml penicillin, and 10 % new born calf serum.

2.2 Spontaneous ^3H—TdR incorporation test of bone marrow cells and thymocytes

Mice were sacrificed by decapitation and the femurs and thymus were immediately excised under aseptic condition. Bone marrow cells of the femurs were flushed out with RPMI 1640 using a syringe and a 25 gauge needle. Thymocytes were obtained by squeezing the thymus with forceps. Finally the cells were dispersed through a 25 gauge needle to give a suspension of single cells and their concentrations were adjusted with RPMI 1640 to 2×10^6 cells/ml for bone marrow cells and 5×10^6 / ml for thymocytes. To each ml of the cell suspensions 37 kBq of ^3H-TdR was added and cultured in a humidified 5 % CO_2 incubator for 24 h. In order to detect the cpm caused by $^{235}U-UO_2F_2$ remaining in the cells, parallel samples were cultured without ^3H-TdR. After 24 h' culture, the cells of each sample were gathered onto a No.49 glass fibre filter, dried at 50°C, dropped into 5 ml scintillation cocktail (0.4 % PPO and 0.04 % POPOP in xylene), and their radioactivities were determined with a Beckman LS 6800 liquid scintillation counter. Results are expressed as percent relative response (mean ± SD) which is defined as the cpm of cells from $^{235}U-UO_2F_2$ injected mice divided by the mean cpm of cells from control mice and multiplied by 100.

2.3 Spleen T and B lymphocytes proliferation test

Spleen lymphocytes were obtained by squeezing the spleen with forceps in RPMI 1640 under aseptic condition. The cell suspension was allowed to stand by for 5 min to collect the sediment and remove the debris. The cells were washed with RPMI 1640, and resuspended in RPMI 1640 to a concentration of 2×10^6cells/ml. Either 100 μg/ml of mitogenic PHA or 20 μg/ml of LPS was added to the cell samples and triplicates of each sample were cultured in humidified 5% CO_2 incubator at 37°C for 72 h. At the end of 56 h, 37 kBq of ^3H-TdR was added to each tube and the culture was continued for 16 h. Then the cells were gathered and their percent relative responses were determined as described above.

2.4 Assay of IL—1 production in spleen lymphocytes

Spleen lymphocytes at a concentration of 5×10^6 cells/ml with 20 μg/ml of LPS were cultured in humidified atmosphere of 5% CO_2 at 37°C for 24 h. Cells were removed by centrifugation at 2000 rpm for 10 min and the supernatant was purified by passing through a 0.22 μm filter. The IL-1 activity in the supernatant was determined by the thymocyte response method. Briefly, 50 μl of diluted or undiluted supernantant was added to 100 μl of mouse thymocyte suspension containing 1.5×10^6cells and 0.3 μg of ConA and cultured at 37 °C for 72 h. After 56 h, 18.5 kBq of ^{3}H-TdR was added to each tube and the culture was continued for 16 h. Then the cells were collected and the radioactivity was determined and expressed as mean ± SD cpm of triplicates.

2.5 Assay of IL—2 production in spleen lymphocytes

Spleen lymphocytes at a concentration of 5×10^6cells/ml with 10 μg/ml of ConA were cultured for 24 h, 48 h or 72 h. Supernatant of the cultured cells was prepared as described for IL-1 production assay and the IL-2 activity was determined with a cloned murine cytotoxic T-lymphocyte line (CTLL), the proliferation of which is known to be IL-2 dependent[3,4]. 50 μl of 25 % supernatant was added to 100 μl of CTLL cell suspension containing 1×10^4 cells. After the mixture has been cultured for 24 h, 18.5 kBq of ^{3}H-TdR was added to each tube and the culture was continued for 16h. Then the cells were gathered and the radioactivity was determined. Parallel positive and negative controls were run simultaneously.

2.6 Study of the influence of exogenous IL—1 and IL—2 on enriched uranium irradiated spleen B lymphocytes

Spleen B lymphocytes from $^{235}U-UO_2F_2$ treated and untreated mice were resuspended to a concentration of 1.5×10^6 cells/ml in complete RPMI 1640 medium containing 20 μg/ml of LPS. 50 μl of the cell suspensions were distributed in 96-well microtitration plates and 50 μl of various concentrations of IL-1 or IL-2 were added to the cell suspensions in triplicates.

The cells were cultured at 37 °C for 56 h, followed by addition of 37 kBq ^{3}H-TdR and another 16 hours' culture. Then the cells were collected and measured for their radioactivities by liquid scintillation counting. In order to observe the synergic effect of IL-1 and IL-2, a mixture of 1×10^4 u of IL-1 and 2×10^5 u of IL-2 was tested in a similar manner.

The effect of exogenous IL on spleen B lymphocytes obtained from either ^{235}U injected or control mice is expressed as proliferation rate which is defined by the equation: Proliferation rate = [cpm of IL treated lymphocytes − cpm of untreated lymphocytes]/cpm of untreated lymphocytes.

3 EXPERIMENTAL RESULTS

3.1 The effect of $^{235}U-UO_2F_2$ on the proliferation ability of central immune cells

The changes of the proliferation ability of thymocytes and bone marrow cells at different times after an intravenous injection of 20 mg/kg of $^{235}U-UO_2F_2$ is shown in Table 1. The ^3H-TdR incorporation in thymocytes decreased significantly at 24 h after injection, dropped to a lowest value at 48 h, and rose markedly at 72 h though was still lower than control. Only at 48 h after ^{235}U injection, ^3H-TdR incorporation of bone marrow cells diminished significantly.

Table 1

Changes of proliferation ability of thymocytes and bone marrow cells after an intravenous injection of 20 mg/kg of $^{235}U-UO_2F_2$

Time after injection	No. of mice	Proliferation ability Thymocytes	Bone marrow cells
Control	20	100	100
24 h	10	34.7 ± 4.5**	116.6 ± 24.6
48 h	14	7.2 ± 4.5**	78.1 ± 10.8*
72 h	13	55.6 ± 17.6**	91.3 ± 7.4

* and **: Results of t-test between experimental and control groups, $P<0.05$ and $P<0.01$

Table 2

Changes of proliferation ability of spleen T and B lymphocytes after an intravenous injection of 20 mg/kg of $^{235}U-UO_2F_2$

Time after injection	No. of mice	Lymphocyte tr PHA stimulated	ransform ation LPS stimulated
Control	15	100	100
24 h	8	83.2 ± 7.2**	65.9 ± 8.7**
48 h	10	133.7 ± 6.2**	71.1 ± 15.7**
72 h	10	134.8 ± 8.0**	71.4 ± 12.7**

** Results of t-test between experimental and control groups, $P<0.01$

3.2 The effect of enriched ^{235}U on proliferation ability of peripheral immune cells

Table 2 illustrates the effect of $^{235}U-UO_2F_2$ on spleen T and B lymphocytes proliferation. The incorporation of ^3H-TdR in spleen B lymphocytes decreased significantly throughout the period from 24 h to 72 h after ^{235}U injection while the incorporation of ^3H-TdR in T lymphocytes was only significantly decreased at 24 h after injection. At 48 h and 72 h after ^{235}U injection, the ^3H-TdR incorporation in T lymphocytes was even higher than control.

3.3 Effect of enriched ^{235}U on IL—1 production in spleen lymphocytes

As shown in Table 3, for spleen lymphocytes obtained from the experimental mice at various hours after ^{235}U injection, no change of IL-1 activity was detected in the diluted supernatant. However, when the supernatant was undiluted, there was significant decrease of IL-1 activity for the lymphocytes obtained at 24 h and 48 h after ^{235}U injection. When the supernatants were added to the CTLL cells to measure IL-2 activity, the ^3H-TdR uptakes were: 521 ± 41 cpm at 24 h, 489 ± 98 cpm at 48 h and 406 ± 48 cpm at 72 h. Parallel results of negative control (CTLL cells without supernatant or exogenous IL-2) was 490 ± 40 cpm, while that of positive control (CTLL cells with 125 u/ml of exogenous IL-2) was 66006 ± 4533 cpm. Therefore, the spleen lymphocyte supernatant was devoid of IL-2 activity.

3.4 The effect of enriched ^{235}U on IL—2 production of spleen lymphocytes

As can be seen in Table 4, the ConA stimulated IL-2 production in lymphocytes

from control mice was highest after 24 hours' culture with ConA and then dropped rapidly when the culture and stimulation was continued. The IL-2 production in lymphocytes of experimental mice taken at 24 h or 48 h after ^{235}U injection showed no difference from the control mice during the first 24 h ConA stimulation. However, when the ConA stimulation was continued to 48 h or 72 h, the IL-2 production was significantly higher than control mice. Therefore, the change of IL-2 production was different from IL-1, i.e., the IL-2 production was not inhibited by enriched ^{235}U.

Table 3

Changes of IL—1 production in spleen lymphocytes after an intravenous injection of 20 mg/kg of $^{235}U-UO_2F_2$

Time after injection	No. of mice	IL-1 activity / cpm	
		12.5 % supernatant	Undiluted supernatant
Control	10	49567 ± 15056	47244 ± 11399
24 h	6	39579 ± 11074	32559 ± 7087**
48 h	6	35190 ± 9222	33194 ± 9074**
72 h	6	48681 ± 4795	39544 ± 6481

** Results of t-test between experimental and control groups. $P<0.01$

Table 4

Change of IL—2 production in spleen lymphocytes after an intravenous injection of 20 mg/kg of $^{235}U-UO_2F_2$

Time after injection of ^{235}U	No. of mice	IL-2 activity of supernatant culture with ConA (cpm/10^4 CTLL cells) after different times		
		24 h	48 h	72 h
Control	8	14634 ± 8497	1858 ± 1388	816 ± 535
24 h	6	18652 ± 8874	7748 ± 4136**	4337 ± 2518**
48 h	6	14072 ± 1747	6828 ± 4609**	3679 ± 2806**
72 h	6	7483 ± 1747	1109 ± 326	579 ± 233

** Results of t-test between experimental and control groups, $P<0.01$

3.5 The protective action of IL—1 and IL—2 on the damaging effect of enriched ^{235}U on B lymphocytes

As shown in Table 5, a single intravenous injection of enriched ^{235}U inhibited the LPS stimulated incorporation of ^3H-TdR in B lymphocytes even if IL-1 or IL-2 was added during the in vitro incorporation test. However, as can be seen from the proliferation rates, the addition of either IL-1 or IL-2 to the cultured cells increased the ^3H-TdR incorporation to a greater degree in the ^{235}U damaged lymphocytes than in normal lymphocytes. The optimal concentrations of IL-1 and IL-2 were 1×10^4 u/ml and 2.5×10^2 u/ml respectively.

3.6 The synergic effect of exogenous IL—1 and IL—2 on spleen B lymphocytes from $^{235}U-UO_2F_2$ injected mice

As also shown in Table 5, when optimal concentration of IL-1 and optimal

concentration of IL-2 were applied together to the cultured cells from $^{235}U-UO_2F_2$ injected mice, the LPS stimulated spleen B lymphocytes incorporation of ^3H-TdR was higher than either IL-1 or IL-2 was applied alone. The proliferation rate was as high as 0.83.

Table 5

The in vitro protective action of IL—1 or IL—2 on spleen B lymphocytes of mice injected with $^{235}U-UO_2F_2$

Addition of IL		Control group		$^{235}U-UO_2F_2$ group	
Type	Unit/ml	cpm	Proliferation rate	cpm	Proliferation rate
IL-1	0	7951 ± 488	—	3327 ± 220	—
	2×10^4	11765 ± 728	0.48	4895 ± 349	0.48
	1×10^4	11074 ± 888	0.40	5558 ± 524	0.67
	2.5×10^3	7533 ± 1161	0	4528 ± 1128	0.36
	8.3×10^2	9718 ± 741	0.23	4056 ± 796	0.22
IL-2	5.0×10^2	8956 ± 324	0.13	4956 ± 418	0.49
	2.5×10^2	9070 ± 1511	0.14	5017 ± 511	0.51
	1.7×10^2	8822 ± 762	0.11	4514 ± 511	0.36
	5.5×10	9924 ± 860	0.25	4468 ± 214	0.35
IL-1 + IL-2	1×10^4 + 2.5×10^2	10744 ± 621	0.35	6081 ± 455	0.83

4 DISCUSSION

In this study, the cell proliferation ability was measured by the ^3H-TdR incorporation test. Spontaneous ^3H-TdR incorporation in cultured thymocytes is a indicator of DNA synthesis in different primary thymocytes ranging from pre-T to mature T lymphocytes. Spontaneous incorporation of ^3H-TdR in cultured bone marrow cells reflects the proliferation ability of primary bone marrow cells which is an indicator of the proliferation ability of lymphocyte stem cells. Results of this work show that enriched ^{235}U is able to damage the function of thymocytes and inhibit the maturation process of T lymphocytes, whereas the bone marrow cells are less affected.

T and B lymphocytes are the most radiosensitive cells. As a whole, B lymphocytes are more radiosensitive than T lymphocytes as can be seen from the comparisons of their survival rates as well as their morphological and functional changes[5,6]. In this study, it was found that the LPS stimulated B lymphocytes transformation was markedly inhibited throughout the 72 hours' period after ^{235}U injection, whereas only a mild inhibition of the PHA stimulated T lymphocyte transformation was observable at 24 h after ^{235}U injection. At 48 h and 72 h after ^{235}U injection, the ^3H-TdR incorporation rate was even higher than normal. It is probale that this is due to the diverse radiosensitivity of the T lymphocyte subpopulations in thymus.

It is well known that IL-1 and IL-2 have multiple immuno-enhancing properties[7,8]. The results presented in this paper reveal that injection of enriched ^{235}U inhibited the IL-1 but not the IL-2 production of spleen lymphocytes. The fact that IL-2 production was not inhibited is probably due to the suppression of lymphocyte proliferation and the decrease of IL-2 consumption[9,10]. In addition, it should be noted that exogenous IL-1 and IL-2 when added to cultured cells, are effective in restoring the decreased proliferation ability of spleen B lymphocytes caused by enriched ^{235}U.

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