THE EFFECTS OF THE ENRICHED URANIUM OXYFLUORIDE ON DNA DAMAGE REPAIR IN MOUSE LYMPHOCYTES *IN VIVO*

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ABSTRACT The paper is to elucidate the effects of in vivo exposure to different levels of $^{235}UO_2F_2$ on DNA repair capability and the mechanism. The DNA repair capacity, as measured by UV-induced unscheduled DNA synthesis (UDS) of splenic lymphocytes from inbred BALB/c male mice, was observed after injection of different doses of $^{235}UO_2F_2$ into the caudal vein. UV-induced UDS of the splenic lymphocytes increased significantly ($p<0.05$ or $p<0.01$) at doses of 0.1—20 μg / kg body weight. Non-UV-induced UDS showed a significant increase ($p<0.05$ or $p<0.01$) 12 days after $^{235}UO_2F_2$ injection. The UV-induced UDS in PHA activated but non-proliferating (hydroxyurea treated) lymphocytes were lower than those of lymphocytes unexposed to PHA at the same doses of $^{235}UO_2F_2$ ($p<0.05$ or $p<0.01$). Low doses of internally deposited $^{235}UO_2F_2$ have a continuous DNA damage effect on the mouse lymphocytes, so that a significant stimulative effect on DNA repair capability in the cells is induced. The stimulative effect occurs only in a limited dose range, and the dose range of internally deposited $^{235}UO_2F_2$ is larger than that of the external radiation. The DNA repair synthesis is significantly inhibited after UV-irradiation in PHA stimulated but non-proliferating lymphocytes.
KEYWORDS $^{235}UO_2F_2$, Lymphocytes, Unscheduled DNA synthesis, Stimulatory effect
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1 Introduction

DNA is very sensitive to a lot of environmental substances such as ionizing radiation or chemical compounds that might induce cancer, teratogenesis and mutation. We have previously observed that enriched uranium oxyfluoride ($^{235}UO_2F_2$) could induce DNA single and double strand breaks. DNA is an important target molecule for ionizing radiation. Radiation-induced DNA strand breaks are repaired efficiently in proliferating mammalian cells, non-dividing cells e.g. hepatocytes, cerebellum cells and various lymphatic cells. DNA excision repair is the main pathway by which mammalian cells remove carcinogenic DNA lesions[1]. The DNA repair is independent on the stage of cell-cycle. DNA synthesis inhibitor such as hydroxyurea does not affect the DNA excision repair. So it is labeled as Unscheduled DNA Synthesis (UDS)[2]. Non-repair or incorrect repair of DNA damage will affect the function of cells. The effect of various damaging agents on UDS in mammalian cells has been studied extensively. Due to the extensive application of $^{235}UO_2F_2$, the biological effect of $^{235}UO_2F_2$, which is closely related to human's living environment, especially its effect on DNA excision repair function in immune cells has not been reported yet. In the present investigation, the ultraviolet light (UV)-induced UDS, by incorporation in vitro of ^{3}H-TdR into DNA of isolated splenic lymphocytes of mice, was measured to study the effects of different doses of $^{235}UO_2F_2$ on the DNA excision repair of mouse lymphocytes.

2 Materials and methods

2.1 Experiment animals

Male BALB/c mice from the Center of Experimental Animal of Soochow University, aged 7—8 weeks and weighed (21±3)g, were used in the experiment.

2.2 Internal contamination of $^{235}UO_2F_2$

A concentration of 18.9 % $^{235}UO_2F_2$ (60g/L) were diluted with injection water. The BALB/c mice were injected through the tail vein with

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0, 0.001, 0.01, 0.1, 1, 20, 100 and 500μg $^{235}UO_2F_2$/kg weight.

2.3 UV-induced UDS assay of the mouse spleen lymphocytes

The mouse spleens were removed with aseptic technique on the 12d after injection and teased apart to release splenic cells in RPMI 1640 medium. After centrifugation at 2000r/min for 10 min, the splenic cells were resuspended at 1×10^7 cells/mL in RPMI 1640 medium containing penicillin (100IU mL^{-1}), streptomycin (100μg mL^{-1}), 10% foetal calf serum and 10^{-2} mol/L hydroxyurea. The cell samples were respectively dispensed into 24-well microplate. Each cell sample occupied 5 wells, 0.1mL of cell suspension each well. The two microplates containing the same cell samples were preincubated in a CO_2 incubator at 37°C for 30 min, 37kBq ^3H-TdR was added to each well.100μL of 200μg mL^{-1}/mL PHA was added to the No.3～4 wells per cell sample per microplate. One microplate was irradiated by 20J/M^2 of UV-ray and the other microplate was not irradiated as a control. Then the two plates were incubated at 37°C under 5% CO_2 for 120min. At the end of incubation, the cells were immediately collected on glass fiber filters by a cell autoharvester. The filters were dried and counted in the Beckman LS 6800 liquid scintillator. The average count-per-minute values of five samples of the controls were subtracted from that of the corresponding (UV)-irradiated samples. The results were expressed as 10^6/ min^{-1} cells.

3 Results

3.1 Effect of $^{235}UO_2F_2$ on UV-induced UDS of the mouse spleen lymphocytes

The effects of different doses of $^{235}UO_2F_2$ on UV-induced UDS of the mouse splenic lymphocytes are shown in Table 1. The UV-induced UDS values were significantly increased ($p<0.05$ or $p<0.01$) when the mice were exposed to $^{235}UO_2F_2$ at doses of 0.1～20μg/kg body weight. The results showed that low-doses of $^{235}UO_2F_2$ had obvious stimulatory effect on DNA excision repair capacity. The stimulatory effect only occurred in a limited range of doses of $^{235}UO_2F_2$.

Tab.1 Effect of $^{235}UO_2F_2$ on UV-induced UDS of the mouse splenic lymphocytes on the 12d ($\bar{x}\pm s$) n=5

Doses /μg kg^{-1}	Counts/min^{-1} /10^6cells	Relative value
0	143.50±55.07	1.00
10^{-3}	133.43±48.52	0.93
10^{-2}	215.48±49.65	1.50
10^{-1}	280.00±38.44	1.95 (2)
1	244.75±44.97	1.71 (1)
20	225.50±38.60	1.57 (1)
10^2	173.50±60.82	1.21
5×10^2	124.00±69.30	0.86

(1) $p<0.05$, (2) $p<0.01$

According to the table 1, a equation of the dose-effect relationship for the UV-induced UDS of the mouse splenic cells after *in vivo* $^{235}UO_2F_2$ contamination was as follows.

$$E = 207-0.299D+0.000264D^2$$

Where E is the UV-induced UDS values of the mouse splenic cell/10^5 min^{-1}), D is the doses(μg) of internal contamination of $^{235}UO_2F_2$.

3.2 Effect of $^{235}UO_2F_2$ on UDS of un-radiated mouse splenic lymphocytes

The effects of $^{235}UO_2F_2$ on the UDS of mouse splenic cells which were not radiated are shown in Table2. The UDS of the splenic cells of the experimental groups showed a pronounced increase ($p<0.05$, $p<0.01$ or $p<0.001$) on the 12d after internal contamination of

Tab.2 The UDS values of the mouse splenic lymphocytes on the 12th day after internal contamination of $^{235}UO_2F_2$

($\bar{x}\pm s$) n=5

Doses /μg·kg^{-1}	Counts/min^{-1} /10^6 cells	Relative value
0	183.50±20.51	1.00
10^{-3}	250.08±60.35	1.36 (1)
10^{-2}	240.88±37.10	1.31 (1)
10^{-1}	269.00±10.46	1.47 (2)
1	232.25±14.86	1.27 (2)
20	236.00±42.15	1.29 (1)
10^2	235.13±26.65	1.28 (2)
5×10^2	264.00±45.59	1.44 (2)

(1) $p<0.05$, (2) $p<0.01$

$^{235}UO_2F_2$. The results indicated that $^{235}UO_2F_2$ *in vivo* had continuous damaging effect on the splenic cells, thereby leading to enhancement of DNA excision repair capacity.

3.3 Effect of PHA on UV-induced UDS of the mouse splenic lymphocytes after $^{235}UO_2F_2$ internal contamination

The mitogen PHA could induce in vitro the differentiation and proliferation of lymphocytes, and led to the increase of semi-conservative DNA replication in the cells. There were higher UV-induced UDS values in the cycling lymphocytes than in resting ones[3]. In order to approach the effects of PHA on UV-induced UDS of splenic lymphocytes after exposure to different doses of $^{235}UO_2F_2$, the variation of UV-induced UDS of PHA-stimulated, but non- proliferating (hydroxyurea-treated) mouse spleen lympho- cytes on the 12d after $^{235}UO_2F_2$ internal contamination was investigated (Table 3). The results showed that the UV-induced UDS values were significantly decreased in PHA-stimulated, but non-proliferating cells than in corresponding unstimulated cells (p<0.05 or p< 0.01).

Tab.3 Inhibitory effect of PHA on UV-induced UDS of the mouse splenic lymphocytes on the 12d after internal contamination of $^{235}UO_2F_2$　　($\bar{x}\pm s$) n=5

Doses / μg kg^{-1}	Counts/min^{-1} / 10^6 cells non-PHA	PHA	PHA non-PHA
10^{-3}	133.43±48.52	36.25±35.71	0.27(1)
10^{-2}	215.48±49.65	38.67±20.28	0.18(2)
10^{-1}	280.00±38.44	181.67±66.25	0.65(1)
1	244.75±44.97	156.57±29.48	0.64(1)
20	225.50±38.60	146.34±40.70	0.65(1)
10^2	173.50±60.82	66.00±28.28	0.38(1)

(1) p<0.05, (2) p<0.01

4 Discussion

4.1 Stimulatory effect of $^{235}UO_2F_2$ *in vivo* on UV-induced UDS of the mouse splenic lymphocytes

$^{235}UO_2F_2$ is a high-LET α-ray radioactive nuclide and possesses the double toxicity as a radiation and a chemical agent to the organism. The soluble $^{235}UO_2F_2$ could be rapidly distributed to kidney, bone, liver and spleen after injection through the tail vein. In our previous study, we found that the DNA repair capability in the mouse splenic lymphocytes were significantly decreased when a large dose of 20mg/kg weight of $^{235}UO_2F_2$ was intaked[4]。In this study the results of Table1 suggested that low-doses of 0.1—20 μg $^{235}UO_2F_2$/kg weight could increase DNA excision repair capacity, i.e. low doses of $^{235}UO_2F_2$ had significantly stimulatory effects on UV-induced UDS of the mouse splenic lymphocytes. In addition the DNA synthesis of PHA-stimulated splenic T lymphocytes was also increased significantly (p<0.05) after intake of $^{235}UO_2F_2$ at a dose of 0.1μg/kg weight as compared with the control[5]. These results indicated low-doses of $^{235}UO_2F_2$ might have an obvious stimulatory effect on DNA semiconservative replication and DNA repair in splenic lymphocytes, and the stimulatory effect occurred only at a limited dose range of $^{235}UO_2F_2$. The dose range was more larger as compared with that for the external radiation[6].

4.2 The continuing effect of $^{235}UO_2F_2$ on DNA damage and excision repair function in splenic lymphocytes

UV-induced UDS values of the mouse splenic lymphocytes were still higher on the 12d in the $^{235}UO_2F_2$ groups than in the control group (p<0.05, p<0.01 or p<0.001) (Table 2). The results suggested that the DNA damage and the excision repair of splenic cells had not yet recovered to normal due to the continuing effect of *in vivo* $^{235}UO_2F_2$. The results were different from the biological effect of external irradiation. The X or γ-ray irradiation-induced DNA strand breaks in lymphocytes are repaired almost completely within 12h. The overwhelming majority of DNA single strand breaks is repaired within 1～2h[7]. In this study the non-UV-induced UDS values were still increased significantly on the 12d after $^{235}UO_2F_2$ exposure.

It was suggested that except for the excretion of 70%～80% $^{235}UO_2F_2$ by urine after 24h of the intake, the remainder of the $^{235}UO_2F_2$ was excreted very slowly and continuously resulted in DNA damage, which led to the enhancement of the DNA repair capacity in the splenic lymphocytes[8]. Owing to the identical dose of the UV-irradiation, the extent of the second damage for DNA induced by UV was the same. Therefor the UV-induced UDS values in the $^{235}UO_2F_2$ group represented the effect of $^{235}UO_2F_2$ on UDS of the mouse splenic lymphocytes, indicating that the low doses of $^{235}UO_2F_2$ could induce the increase of the DNA excision repair capacity in the splenic lymphocytes.

4.3 The inhibitory effect of PHA on the UV-induced UDS in the splenic lymphocytes after $^{235}UO_2F_2$ internal contamination

The level of UV-induced DNA repair synthesis was approximately tenfold higher, and DNA strand breaks were repaired more rapidly in PHA-stimulated lymphocytes than that in PHA unstimulated cells [3]. In the present investigation, the level of UV-induced UDS was lower in PHA-stimulated, but nonproliferating lymphocytes than that in PHA-unstimulated lymphocytes treated with the same dose of $^{235}UO_2F_2$. The results suggested that PHA had an obviously inhibitory effect on UV-induced UDS of splenic lymphocytes after $^{235}UO_2F_2$ intake. Due to treatment of hydoxyurea, PHA could only be in combination with the corresponding acceptor of the lymphocytes and stimulate the lymphocytes, but could not proliferate the cells. The effect of PHA on lymphocytes might partly consume ATP in the cells. The repair of DNA strand breaks requires ATP. A loss of ATP might be one of the reasons for the reduction in DNA repair capacity.

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浓缩铀内污染对小鼠脾淋巴细胞 DNA 损伤修复的作用

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摘要　研究了不同水平浓缩铀（$^{235}UO_2F_2$）对淋巴细胞 DNA 损伤修复的作用及其机理。应用紫外线诱导的非程序 DNA 合成（UDS）检测，观察浓缩铀（$^{235}UO_2F_2$）内污染对小鼠脾淋巴细胞 DNA 损伤修复的影响。结果表明，浓缩铀摄入量为 0.1—20μg/kg 体重时，脾淋巴细胞紫外线诱导的 UDS 显著高于对照组（$p<0.05$ 或 $p<0.01$）；浓缩铀内污染 12d 时，脾淋巴细胞未经紫外线照射的 UDS 显著增加（$p<0.05$ 或 $p<0.01$）；PHA 组脾淋巴细胞紫外线诱导的 UDS 显著低于未加 PHA 组。在一定剂量范围内，低剂量浓缩铀内污染对淋巴细胞 DNA 损伤修复功能具有明显的刺激作用，并且该剂量范围明显大于外照射；浓缩铀内污染对淋巴细胞 DNA 具有持续的损伤作用，继而诱发细胞 DNA 修复功能的增强；PHA 刺激但未增殖的脾淋巴细胞 DNA 损伤修复功能明显减低。

关键词　浓缩铀，淋巴细胞，非程序 DNA 合成，刺激作用

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