INDUCTION OF MUTAGENIC EFFECT IN SOMATIC AND GERM CELLS BY ENRICHED URANIUM*

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ABSTRACT

Enriched uranium(UO_2F_2) accumulated in organism could cause chromosome aberrations in somatic cells, its rates on bone marrow cells were elevated when the dose of $^{235}UO_2F_2$ was increased. Among the types of induced aberrations, chromatid breakage was predominant, accompanied with a few chromosome breakage and translocation. At the same time mitosis index of metaphase cells was depressed. Chromatid delation and chromatid exchange were induced in peripheral blood lymphocytes. The important type of aberrations in spermatogonia was break. For primary spermatocytes the most significant aberration was multivalents which resulted either from chromatid interchanges or reciprocal translocations. $^{235}UO_2F_2$ could result in DNA breakage in germ cells. The sensitivity of germ cells at various stages to $^{235}UO_2F_2$ was different. At 12 d after exposure the amount of sperm DNA eluted reached the peak. When the treating time was fixed, elution of sperm DNA from treated animals increased with the increasing doses. $^{235}UO_2F_2$ could also result in sperm abnormalities. Especially at 13 to 36 d after treatment the rates of sperm abnormalities were significantly elevated.

Keywords: Enriched uranium Somatic cells Germ cells Chromosome aberrations DNA Mutagenic effect

1 INTRODUCTION

Enriched uranium can be applied to nuclear fuel as well as nuclear weapon, so we paid attention to its metabolic peculiarity in organism[1,2]. It is necessary to study on radiogenotoxicology of enriched uranium deeply[3]. In the environment of nuclear fuel production, soluble and insoluble enriched uranium could contaminate the body by different ways[4,5]. Its action and injury effect, particularly its mutagenic effect in the body showed a close relation on retentive peculiarity of enriched uranium[6]. But concerned studies were not reported yet. For this reason, we studied the retentive

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peculiarity of enriched uranium on mutagenic effect in somatic and germ cells.

2 EXPERIMENTAL METHODS AND RESULTS

2.1 Mutagenic effect in somatic cells by enriched uranium

2.1.1 Chromosomal aberrations and the depressed effect of cell division in bone marrow cells

UO_2F_2 with 18.9 % of ^{235}U was used in this study. Sexually mature males Wistar rats of 188± 12 g were randomly divided into 3 groups: The two experimental were treated with single i.v. injection of $^{235}UO_2F_2$ 20 mg/kg and 30 mg/kg each, and one control. Internal contaminated rats were decapitated after 24 h. Samples of femur were obtained quickly from the sacrificed animals. Bone marrow cells were suspended in RPMI 1640 solution, adjusted to 1.5×10^6 cells/ml. They were cultured in a jar with 5 ml RPMI 1640 solution, incubated at 37 °C for 24 h. After that one drop of 1 % colchicine was added to the end concentration about 1×10^6 mol/ml. Then incubated continually for 4–6 h, and centrifuged at 1000 r/min for 5 min. Take away the supernatant. The bone marrow cells were incubated at 37 °C for 1 h in 75 mmol/l KCl hypotonic solution, then fixed, dispensed on to slides and stained with Wright solution. Metaphase cells were analysed for chromosomal aberrations (see Table 1). Results indicated that there was a positive correlation between the chromosome aberration rates and the amount of intake of ^{235}U, and the mitosis index of metaphase induced were lower when treated with 20 mg/kg and 30 mg/kg in comparison with control. Among the types of chromosome aberrations induced, chromatid breakage were predominant. Chromatid breakage induced by i.v. enriched uranium and chromatid exchange are shown in Fig. 1 and 2, respectively.

Table 1

Chromosome aberrations and mitosis index of metaphase induced in bone marrow cells by i.v. enriched uranium with different doses after 24 h

Group	No. of rats	Chromosome aberrations: Mitosis metaphase No. of MPS	Type of aberrations CMB	Gap	CSB	Abnormal/ %	*P*	Mitosis index of metaphase: No. of MPS	Mitosis meta-phase	Mitosis index/ %	*P*
Control	7	1089	4	2	0	0.55±0.22	–	14259	702	4.92±0.25	–
20 mg/kg	10	1720	44	31	0	4.36±0.49	<0.01	12900	496	3.84±0.73	<0.05
30 mg/kg	10	1587	43	44	0	5.48±0.57	<0.01	11700	415	3.35±0.82	<0.05

Notice: MPS: Metaphase scored CMB: Chromatid breakage CSB: Chromosome breakage

2.1.2 Chromosomal aberrations and the depressed effect of cell division in peripheral blood lymphocytes

Experiments were carried out on 35 sexually mature male Wistar rats of 190±10 g.

They were also randomly divided into two experimental groups and one control. The injection doses and sample's preparation were the same as in the former. Both chromatid breakage and chromatid exchange are showed in Fig.3 and 4 respectively. At the same time the mitosis index of metaphase induced was depressed (see Table 2).

Table 2

Aberrations and mitosis index of metaphase in peripheral blood lymphocytes by i.v. $^{235}UO_2F_2$ after 24 h exposure

Groups	No. of rats	Time of incubation /h	Time of exposure /h	No. of metaphase scored	Mitosis metaphase Type of aberrations: chromatid breakage	Chromatid exchange	Gap	Chromosome breakage	Abnormal /%	P
Control	13	72	24	1829	7	0	6	0	0.71±0.19	-
20 mg/kg	10	72	24	1148	10	0	15	0	2.18±0.43	<0.05
30 mg/kg	11	72	24	1071	22	3	19	1	4.20±0.61	<0.05

Groups	No. of rats	Time of incubation /h	Time of exposure /h	No. of metaphase scored	Mitosis metaphase	Mitosis index	P
Control	15	72	24	22717	3887	17.12±4.14	-
20 mg/kg	10	72	24	12484	930	7.45±0.24	<0.01
30 mg/kg	10	72	24	13148	866	6.59±0.22	<0.01

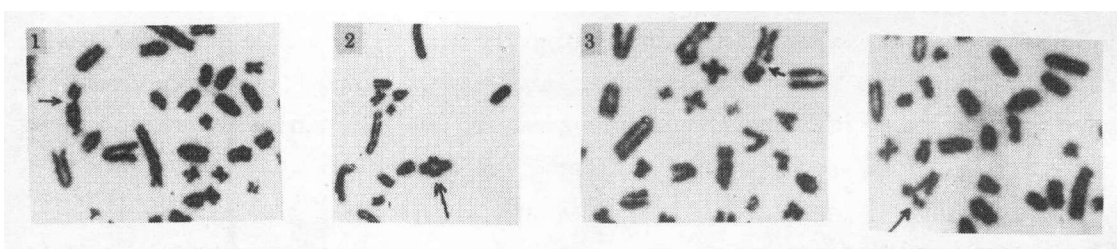

Fig.1—4 Chromatid breakage (1,3) induced by i.v. $^{235}UO_2F_2$ 20 mg/kg and chromatid exchange (2,4) induced by i.v. $^{235}UO_2F_2$ 30 mg/kg all after 24 h using Wright stain in bone marrow cells and peripheral blood lymphocytes respectively

2.2 Mutagenic effect in germ cells by enriched uranium

2.2.1 Chromosomal aberrations in spermatogonia and primary spermatocytes

Sexually mature male BALB/c strain mice of 25± 1 g were used. The time from treatment to chromosome preparation were fixed at 1, 13, 36 and 60 d, respectively, to investigate chromosome aberrations by $^{235}UO_2F_2$ at different stages of germ-cell development. There were 5 experimental groups, treated with i.t. injections of different doses of $^{235}UO_2F_2$ ranging from 0.05 to 1 μg/testis. A control group was treated with isotonic saline in the same way for each fixation time. Each group included 5 males. The volume of $^{235}UO_2F_2$ solution or isotonic saline injected into each testis was 5 μl.

Colchicine of 4 mg/kg was injected i.p. 5.5 h before killed. Chromosome specimens were prepared from the testis according to Ref.[7] with minor modification, and stained with Giemsa in phosphate buffer (pH 6.8). One hundred well-spread metaphase spermatogonia and about the same number of primary spermatocytes per animal were analyzed under the microscope.

Gaps were defined as achromatic lesions smaller than the width of the chromatid and without dislocation of the distal segment. Breaks were scored when the achromatic region was accompanied by displacement of the distal segment. The form of multivalents in meiosis I depends on the number of chromosome involved in pairing, the number and location of chiasmata, and the degree of chiasma termination. In this study multivalents observed at metaphase I were the result of either a chromatid interchange induced in diplotene or preleptotene of meiosis or a reciprocal chromosome-type exchange induced in spermatogonia. The chromatid interchanges were mostly irregular and non-symmetrical configurations. The chromosome-type exchanges were reciprocal translocation in which they were symmetrical configurations.

The variance test was used to check the data homogeneity. When heterogeneity of the variance was found arcsine transformation was carried out before Dunnett's t-test was used to determine statistically significant differences between treatment and control groups.

$^{235}UO_2F_2$ did not significantly induce gaps although there was a slight increase, compared with the control, in gap number in treatment groups, especially at 1 d fixation time. Breaks were found to increase with increasing doses at 4 fixation times and their frequencies were statistically significant in high-dose groups at 1, 13 and 36 d. The frequencies of breaks are more meaningful in cytogenetic evaluation than the other criteria. Since either gaps or polyploids are no good indicators of chromosome damage according to the general opinion[8,9]. In treated groups more breaks were found at the 1 d than at other fixation times. There is no other explanation but prolonged exposure to ^{235}U deposited in testis, for the significant increase in break yields at 13 and 36 d (Fig.5). $^{235}UO_2F_2$ induced fragments, univalents and multivalents. The XY or autosome univalent frequency did not significantly increase with dose. The frequency of fragments only increased at the 1 d. It was noted that all multivalents observed in this study were the same as 'quadrivalents' (Fig,6). They were presented in the form of either a ring or a chain. The frequency of chromatid interchanges significantly increased at the 13 d. The reason for this may be that cells in preleptotene at the time of treatment were the most likely ones to show aberrations according to Preston et al[10]. No significantly increased production of reciprocal translocations was observed compared with the control. Cells with univalents were not included in the category of aberrant cells because rather high

frequencies of univalents were found in control groups.

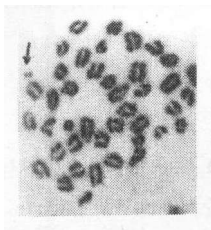

Fig.5 Chromosome breakage induced in spermatogonia by i.t. $^{235}UO_2F_2$ 0.5 μg after 13 d. Giemsa stain, ×960

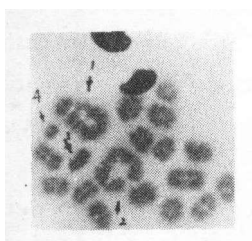

Fig.6 Quadrivalent in the form of a ring induced in primary spermatocytes by i.t. $^{235}UO_2F_2$ 1 μg after 60 d

Giemsa stain, ×1200

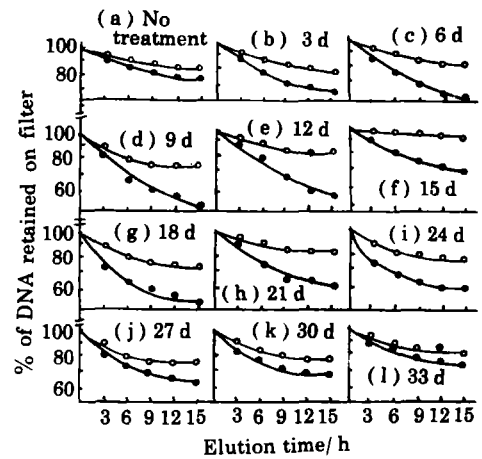

Fig.7 Alkaline elution profiles of sperm DNA from animals treated with either no $^{235}UO_2F_2$(a), or 2 mg/kg $^{235}UO_2F_2$ by i.p. injection at different times before assay(b—l)

○ ^{14}C-DNA from internal control

○ ^{3}H-DNA from external control or treated mice

Each elution curve represents the average of seperate elution runs

2.2.2 Detection of DNA damage in spermiogenic stages

Adult male BALB/c strain mice of 24±1 g were randomly divided into 17 groups of 4 males each: one external control, 11 time–experiment and 5 dose–experiment groups. In the external control and each time–experiment group, 2 males were given i.t. injections of ^{3}H–TdR of 167 kBq/testis and the other two of ^{14}C–TdR of 52 kBq/testis for sperm DNA labeling using procedures described by Sega *et al*[11]. In dose–experiment groups the sperm DNA of all mice was labeled with ^{3}H–TdR using the same way. The animals receiving i.t. injection of ^{14}C–TdR served as internal controls[12]. Animals with ^{3}H–TdR labeled sperm DNA except the external control were to be treated with $^{235}UO_2F_2$. At different times following i.t. injection of labeled thymidine, the to–be–treated animals were given i.p. injection of 2 mg $^{235}UO_2F_2$/kg for treatment. The time of sperm recovery from the treated mice was from 3 to 33 d after treatment at 3–day intervals because maximum labeling of spermatozoa recovered from the vas of labeled males with ^{3}H–TdR or ^{14}C–TdR had been found by Sega *et al*[11]. Dose experiments were made using 5 different exposures of $^{235}UO_2F_2$ from 0.5 to 6.0 mg/kg. In these experiments the time from treatment to assay was fixed at 12 d

because we had found that at this time the eluted sperm DNA from mice treated with 2 mg/kg $^{235}UO_2F_2$ was more than that at other times.

Preparation of sperm cells Experimental mice were killed at 36 d after radioactive thymidine labeling. Each alkaline elution consumed one treated mouse (^{3}H-TdR labeled) and one internal control mouse(^{14}C-TdR labeled) for time experiment or simply one treated mouse for dose experiment. The vasa from each animal were removed and placed in 5 ml phosphate-buffers saline(PBS) at pH 7.2 in the dark to prevent light damage to sperm DNA. In 15 min most of the sperm swam out of the vas into the PBS. Remaining sperm clumps were distangled by gentle pipetting with a pipette after the vas was removed from the PBS. This procedure results in very little trauma to the sperm and sperm motility at this point is high. It is especially important that the number of sperm cells swimming into the 5 ml PBS be larger than 5×10^6 and thus enough to be assayed.

Alkaline elution Cell aliquot containing 5×10^6 cells was gently applied onto 2-μm pore size polyvinyl chloride filter (25 mm. Millipore Corp., USA) placed inside a plastic filter holder and attached to a 50-ml glass syringe barrel. Sperm cells were lysed by filling the syringe barrel with 5 ml lysis solution of 0.025 mol/l disodium ethylenediamine tetraacetic acid, 2% high purity sodium dodecyl sulfate(SDS), 1.0 mg/ml freshly dissolved cysteine and 0.5 mg/ml freshly dissolved proteinase K adjusted to pH 9.6. All the lysis solution but the last milliliter was drained through the filter. After 40 min at room temperature, the last milliliter of lysis solution was allowed to drip out. Then sperm lysis was completed. The filter was eluted in the dark at a flow rate of 40 μl/min, by a peristalic movement pump, using 40 ml of eluting buffer containing 2% TPAH adjusted to pH 12.2, 0.02 mol/l acid EDTA and 0.1% high purity SDS. Fractions were collected at 3 h intervals for 15 h.

Measurement of DNA eluted Two ml solution was blotted from each elute DNA fraction onto a glass fiber filter(25 mm). The glass fiber filters together with the polyvinyl chloride filter initially placed inside the filter holder were dried and placed each in a scintillation vial. Five ml of a scintillation mixture, consisting of 100% toluene, 0.6% PPO, and 0.03% POPOP was added and the radioactivities of ^{3}H- and ^{14}C-DNA were determined by liquid scintillation counting.

Results of time experiment The time course of alkaline elution of sperm DNA recovered from animals receiving either no $^{235}UO_2F_2$(Fig.7a) or 2 mg $^{235}UO_2F_2$/kg at different times before assay(Fig.7b— l) is shown in Fig.7. By 3 d after treatment (Fig.7b), sperm DNA elution of treated animals was increased apparently. By 12 d the amount of treated sperm DNA eluted was the greatest and after 21 d it was progressively reduced.

Results of dose experiment The relation between the dose of $^{235}UO_2F_2$ given to mice by i.p. injection (0.5— 6.0 mg/ kg) and the amount of the treated sperm DNA

eluted 12 d following treatment may be express closely located around the least square linear fit line: $y=4.99x+28.33$ [y–% of ^{3}H–DNA eluted, x–the dose of $^{235}UO_2F_2$ (mg/kg)]. In this experiment no animals served as internal controls. It was noticed that the elution of the sperm DNA from the external control, at the zero dose, was rather high. Maybe this occured from the labeling tritium in the testis.

2.2.3 Induction of abnormalities in sperm

It was studied by i.t. injection of $^{235}UO_2F_2$ to sexually mature male BALB/c strain mice of 25 ± 1 g. The times from treatment to sperm preparation were fixed at 1, 13, 36 and 60 d. There were 5 experimental groups, treated with single i.t. injections of different doses ranging from 0.05 to 1.00 μg/testis. Experimental mice were killed at various time intervals. The sperm duct from each animal was removed and placed in 6 ml PBS at pH 7.2. After 8–10 min, the sperm swam out of the duct in the PBS. Remaining sperms were distangled by gentle pipetting with a pipette after the sperm duct was removed from the PBS. Then drop the PBS to the cleaned microscope slides and dispersed sperm specimen carefully. The slides should then be allowed to drain and dry in a dust–free atmosphere. The sperm specimens were fixed by absolute methylalcohol, Eosin staining for 1 h, then analyzed under microscope(see Table 3).

Table 3

Induction of sperm abnormalities by i.t. injection of $^{235}UO_2F_2$ in different intervals

Dose/	The rates of sperm abnormalities / %			
μg per testis	1 d	13d	36 d	60 d
1.00	9.75±0.66	28.85±1.01* * *	25.20±0.97* * *	12.90±0.75* * *
0.50	9.70±0.66	23.65±0.95* * *	18.35±0.87* * *	10.95±0.70* *
0.25	9.20±0.65	16.60±0.83* * *	16.10±0.82* * *	10.50±0.69
0.10	8.70±0.63	16.10±0.82* * *	14.45±0.78* * *	8.95±0.64
0.05	9.00±0.64	14.35±0.78* * *	13.80±0.77* * *	9.75±0.66
Control	9.20±0.65	8.70±0.63	8.90±0.64	9.00±0.67

* * $P<0.05$, * * * $P<0.01$

Even with the same dose but at different treating time the rates of sperm abnormalities were different. At 1 d there was no significant difference in the rates of sperm abnormalities between treated groups and the control. At 13 d and 36 d the rates in treated groups were significantly higher than those in the controls, while at 60 d the rates in high dose groups were still high as compared with the control.

Regarding to the form of sperm abnormalities, such as biceps, bitails, irregular, and non–hook were likely to be found.

3 DISCUSSION

Mutagenic effects in somatic cells such as in bone marrow cells and peripheral blood lymphocytes were elevated when the UO_2F_2 dose was increased. Internal

contamination of enriched uranium can induce some aberrations in one cell. This phenomenon might be due in part to nonuniform irradiation of bone marrow cells and peripheral blood lymphocytes with local deposition of $^{235}UO_2F_2$.

Results of the experiment show that spermatogonia were sensitive to a trace $^{235}UO_2F_2$. Spermatogonia are important target cells and an elevation of the aberrations induced in spermatogonia is important for estimation of genetic risk since spermatogonia will later on give rise to mature germ cells. But many spermatogonia, carrying more than one aberration after treatment, may be eliminated before reaching the spermatocyte stage. For that reason it is genetically meaningful that the reciprocal translocations induced in spermatogonia are recorded in spermatocytes.

In this study sperms were recovered from vasa deferentia of mice. According to the duration of spermatogenesis in the mouse, sperm recovered at 1, 13, 36 and 60 d were vas sperm, testicular sperm, spermatogonia and stem spermatogonia, respectively. No significant difference between the treated and control groups at 1 d suggests that the amount of enriched uranium reaching vasa deferentia was too small to cause many abnormal sperms. The 13 and 36 d data showed good relationships between the rates of abnormal sperm and doses of enriched uranium, which indicate that $^{235}UO_2F_2$ could result in sperm abnormality after the contact with germ cells at testicular sperm and spermatogonia stages. The 60 d data showed there were low rates of sperm abnormality compared with the 13 and 36 d data, but the rates in high dose groups were still significantly higher than that in the control.

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