Comparative study on metabolic peculiarity in testes and reproductive toxicity induced by different radiators*

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Abstract The fitted equations to describe the retentions of α radiator ^{235}U and β ^{147}Pm as well as γ ^{134}Cs were obtained respectively. The half life component of ^{235}U is the longest, $T_{1/2}$=197 d; of ^{147}Pm is shorter, $T_{1/2}$=76 d; and of ^{134}Cs is the shortest, $T_{1/2}$=5.1 d. For the rates of sperm abnormality induced by 1 cGy cumulative absorption dose the ratio of reproductive toxicity is α:β:γ =28:3:1. For the frequencies of chromosome aberrations in spermatogonia induced by 1 cGy cumulative absorption dose, the ratio of reproductive toxicity is α:β:γ=29:4:1. In the general, reproductive toxicity of α radiator ^{235}U is the biggest, next β ^{147}Pm, finally γ ^{134}Cs.

Keywords Comparative evaluation, Testes, Retention, Reproductive toxicity, ^{235}U, ^{147}Pm, ^{134}Cs

1 Introduction

Up to date, due to the rapid development of nuclear power station and the wide scope application of radioisotopes, the number of persons contacting with ^{235}U, ^{147}Pm and ^{134}Cs is increasing constantly.[1] In the sphere of radiation medicine, what concerned about is the environmental pollution and damage to human beings[2] by ^{235}U, ^{147}Pm and ^{134}Cs. In this respect, it should be particularly noted study of genetic materials damage such as abnormalities in sperm and chromosome aberrations in spermatogonia induced by different radiators in germ cells. So we investigated metabolic peculiarity in testes as well as reproductive toxicity for α, β and γ radiators.

2 Experimental methods and results

2.1 Retention and dose estimation of ^{235}U, ^{147}Pm and ^{134}Cs in testes

Experiments for ^{235}U were carried out on sexually mature male BALB/c strain mice of 20±2 g. Animals were randomly divided into 4 groups. Uranyl fluoride containing ^{235}U of 18.9% (60 mg/ml) was used in this study. The transference and accumulative peculiarities of ^{235}U were observed after i.v. 42.6 Bq/g (20μg/g). Mice were killed by decapitation 1, 2, 4, and 8 d after i.v. ^{235}U. Samples of testes were obtained from the sacrificed animals. Each sample of 50 mg was prepared in the form of homogeneous clear solution by adding 0.1 ml $HClO_4$ and 0.2 ml 30% H_2O_2 in a scintillation vial, then put it into the water bath at 80°C for 1 h and add 5 ml ethylene glycol ether to the above solution[3] after cold. At last 8 ml of scintillation mixture consisting of 100% toluene and 0.6% PPO was added. Radioactivities of α emitter samples were determined by liquid scintillation counting with the aid of a Beckman LS 6800. (see Table 1)

Table 1 Retention of ^{235}U in testes

Time/ d	Number of mice	Absolute retention/Bq	Relative retention
1	5	7.2±0.9	0.0084
2	5	6.8±1.1	0.0081
4	5	6.1±0.8	0.0072
8	5	5.6±0.6	0.0066

A fitted equation to describe the retention of ^{235}U in testes is obtained as follows:

$$R(t) = 0.0558\exp(-0.0035t) \quad (1)$$

where $T_{1/2}$ is 197 d.

According to the effective retentive fraction $R(t)$, with respect to time of accumulation 30 d, the cumulative activities $A(t)$ in Bq of ^{235}U in testes were obtained:

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$$A(t) = \int_0^t A_0 R(t)\mathrm{d}t \qquad (2)$$

here A_0 is the early radioactivities of ^{235}U in testes.

Again from the cumulative radioactivities of ^{235}U in testes through 30 d the cumulative absorption doses (CAD) [4,5] can be calculated and shown in Table 2.

Similarly, after injection of 185 kBq/g ^{147}Pm or 46.3 kBq/g ^{134}Cs their experimental results of dynamic retention are shown in Tables 3 and 5, respectively.

Table 2 CAD in testes after i.v. ^{235}U with different radioactivities through 30 d

Radioactivities/Bq·g^{-1}	CAD/Gy
0.04	9.14×10^{-5}
0.2	4.57×10^{-4}
1.1	2.85×10^{-3}
2.1	4.57×10^{-3}
4.3	9.14×10^{-3}
6.4	1.38×10^{-2}

Table 3 Retention of ^{147}Pm in testes

Time/ d	Number of mice	Absolute retention/kBq	Relative retention
2 h	5	33.9±15.2	0.009
5 d	5	45.8±6.9	0.012
10 d	5	55.8±8.3	0.014
20 d	5	38.1±20.1	0.01
50 d	5	27.4±13.7	0.007

The retentive equations of ^{147}Pm and ^{134}Cs in testes are given in Eq.(3) and Eq.(4), respectively

$$R(t) = 0.0118\exp(-0.0092t) \quad T_{1/2} = 76\,\mathrm{d} \qquad (3)$$

$$R(t) = 0.0048\exp(-0.1372t) \quad T_{1/2} = 5.1\,\mathrm{d} \qquad (4)$$

Based on the cumulative radioactivities of ^{147}Pm and Ref.[6]; and based on cumulative radioactivities of ^{134}Cs and Ref.[7], CADs of β emitter ^{147}Pm and γ emitter ^{134}Cs are calculated and shown in Tables 4 and 6, respectively.

Table 4 CAD in testes after i.v. ^{147}Pm with different radioactivities

Radioactivities/Bq·g^{-1}	CAD/Gy
3.7×10^{2}	0.79×10^{-4}
3.7×10^{3}	7.6×10^{-3}
3.7×10^{4}	7.7×10^{-2}
9.3×10^{4}	2.6×10^{-1}
1.9×10^{5}	1.6

Table 5 Retention of ^{134}Cs in testes

Time/ d	Number of mice	Absolute retention/kBq	Relative retention
10 h	5	4.0±0.4	4.0×10^{-3}
2 d	5	3.8±0.9	3.9×10^{-3}
6 d	5	2.6±0.4	2.8×10^{-3}
15 d	5	0.5±0.1	6×10^{-4}
30 d	5	0.05±0.01	5×10^{-5}
47 d	5	0.01±0.005	1×10^{-5}

Table 6 CAD in testes after i.v. ^{134}Cs with different radioactivities

Radioactivities/Bq·g^{-1}	CAD/Gy
3.7×10^{2}	1.8×10^{-3}
1.9×10^{3}	1.4×10^{-2}
9.3×10^{3}	3.8×10^{-2}
4.6×10^{4}	1.26×10^{-1}
2.3×10^{5}	6.1×10^{-1}

2.2 Abnormalities in sperm

For the experiment induced by ^{235}U, sexually mature male BALB/c strain mice of 25±1 g were randomly divided into 4 experimental groups and corresponding control group. Mice of experimental groups were given i.v. injection of 2.1 Bq/g ^{235}U, and killed 1, 13, 30 and 60 d after the injection. For control ^{235}U was replaced by saline. The sperm duct from each mouse was removed and placed in 10 ml PBS at pH 7.2. The sperm swam out of the duct after 10 min. Remaining sperms were gently pipetted out after the sperm duct was removed from the PBS. Then, drop the PBS to the cleaned microscope slides and disperse sperm specimens carefully. The slides should 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.

Experimental results are shown in Table 7. Table 2 indicates that the CAD of ^{235}U at 30 d interval was 4.57 mGy. Then, abnormalities in

sperm at this 'interval were 25.2%. So abnormalities in sperm induced by ^{235}U of 1 cGy were 55.1%.

Similarly, after i.v. injections of 185 kBq/g ^{147}Pm and 46.3 kBq/g ^{134}Cs, their abnormalities in sperms are observed, the results also shown in Table 7; the abnormalities in sperms induced by ^{147}Pm of 1 cGy or ^{134}Cs of 1 cGy are 5.7% and 1.97%, respectively.

2.3 Chromosome aberrations in spermatogonia

For ^{235}U, its experimental details are the same as the afore said. But, colchicine of 4 μg/g was injected i.p. 5.5 h before killed. Samples of testes were obtained quickly from the sacrificed animals, and put into 10 ml of 1% sodium citrate hypotonic solution. Then samples were removed out of the peripheral adipose tissue, cut away the testis membrane, and isolated the testis convoluted tubules. Thereafter, testis convoluted tubules were collected and incubated at 37°C for 40 min in sodium citrate hypotonic solution, then centrifuged and put away the supernatant. Samples were fixed by solution of absolute methanol: glacial acetic acid=3:1 for 20 min. In order to soften the convoluted tubules 70% glacial acetic acid was added. Finally, well spread metaphase spermatogonia were dispersed on the slides and stained with Giemsa in phosphate buffer (pH 6.8). Metaphase spermatogonia cells were analysed for chromosome aberrations.

Table 7 Abnormalities in sperm induced by different radiators in testes

				^{235}U			
Time after iv	control	1 d		13 d		30 d	60 d
No. of mice	5	5		5		5	5
Abnormality/%	9.2±0.6	9.8±0.7		28.9±1.0**		25.2±1.0**	12.9±0.8**
				^{147}Pm			
Time after iv	control	10 d		30 d		50 d	
No. of mice	5	5		5		5	
Abnormality/%	6.1±1.8	10.5±1.9*		21.9±2.5**		15.7±2.1**	
				^{134}Cs			
Time after iv	control	10 h	2 d	6 d	15 d	30 d	60 d
No. of mice	5	5	5	5	5	5	5
Abnormality /%	8.2±1.9	25.9±4.4**	30.4±2.9**	30.4±6.7**	31.4±11.6**	24.8±5.6**	27.9±3.3**

*P <0.05, **P <0.01

It should be noted that among the types of chromosome aberrations induced by ^{235}U chromosome breakage was predominant as shown in Table 8.

Table 8 Chromosome aberrations (CA) in spermatogonia induced by different radiators in testes

			^{235}U			
Time after iv	control	1 d		13 d	30 d	60 d
No. of mice	5	5		5	5	5
CA/%	0.2±0.2	3.0±0.9**		1.4±0.7*	1.5±0.4**	1.0±0.4*
			^{147}Pm			
Time after iv	control	2 h	5 d	10 d	30 d	60 d
No. of mice	5	5	5	5	5	5
CA/%	0.2±0.01	0.2±0.01	1.4±0.03*	1.4±0.05*	1.6±0.02*	3.25±0.06**
			^{134}Cs			
Time after iv	control	10 h	2 d	15 d	30 d	47 d
No. of mice	5	5	5	5	5	5
CA/%	0.3±0.03	0.4±0.04	2.6±0.10**	1.4±0.07*	1.4±0.07*	0.5± 0.05

*P <0.05, **P <0.01

The CAD of ^{235}U in testes at 30 d interval was 4.57 mGy (see Table 2). While chromosome aberration rates in spermatogonia at corresponding interval were 1.5%. Therefore, chromosome aberration rates induced by ^{235}U of 1 cGy were 3.2%.

In the same way, induced results by ^{147}Pm and ^{134}Cs are obtained, and also shown in Table 8; the chromosome aberration rates induced by ^{147}Pm of 1 cGy and ^{134}Cs of 1 cGy are 0.41% and 0.11%, respectively.

3 Discussion

At present, people are paying close attention to internal contamination of different radiators to human health through ecological environment. Disturbances in sexual function as well as fertility injury induced by radionuclides rose gradually in clinic.[8] Therefore, quantitatively comparative study on reproductive toxicity induced by different radiators become an important task. Our experimental results show that ^{235}U, ^{147}Pm and ^{134}Cs all could damage genetic materials and cause abnormalities in sperm as well as chromosome aberrations in spermatogonia. But induced effect of α radiator ^{235}U is the biggest, next β radiator ^{147}Pm and finally γ radiator ^{134}Cs.

It should be noted the dynamic courses of transportation and retention by different radiators internal contaminations determine the cumulative absorption dose in testes.[9] So understanding of the dynamic law of different radiator internal contaminations is the basis of explaining the action of different radionuclides in germ cells.

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