STIMULATING EFFECT OF LOW DOSE ^{147}Pm ON DNA REPAIR IN SPERMIOGENIC STAGES*

Zhu Shoupeng (朱寿彭) and Wang Liuyi (王六一)

(*Faculty of Radiological Medicine, Suzhou Medical College, Suzhou 215007, China*)

ABSTRACT

For labelling spermatid DNA, male BALB/c mice are injected intratesticularly with ^{3}H-TdR in advance. After 36 d of labelling, spermatozoa are sampled which is just the time required for the germ cells to mature. The period from intraperitoneal injection of ^{147}Pm to sampling spermatozoa is 12 d. After sperm cells have been lysed, 30 ml eluent is added by wriggle pump. Finally, both the DNA remained on the filters and filtrated bottles are determined with a Beckman liquid scintillation device. Results show that after small dose internal irradiation with 185 Bq/g of ^{147}Pm, an increase in amount of sperm DNA on the filter observed is considerably higher than the control group. This indicates that low level internal radiation of ^{147}Pm has tendency to stimulate the DNA repair in spermiogenic stages.

Keywords Stimulating effect, ^{147}Pm, Low dose irradiation, DNA repair, Spermiogenic stages, Internal irradiation, Mice

1 INTRODUCTION

The largest part of previous research was directed towards high irradiation dose effects[1]. During recent years, remarkable new advances in molecular biology and biochemical as well as cellular genetics have been made. Application of this new understanding and these new techniques to low dose irradiation effects were mostly restricted to external irradiation. For certain biological systems there were some evidences that beneficial effects might result from low dose exposures[2].

^{147}Pm, one of the abundant by-products of nuclear industry, has been used as energy sources in luminescent paints for watches and instrument dials. Separation of ^{147}Pm and its preparation for industrial applications constitute a potential radiation exposure to workers in this field. We have studied the transference and accumulation of ^{147}Pm in cellular and subcellular level[3] as well as its action and injury effects[4] in organism, but there seems no report on stimulating effect of low dose ^{147}Pm internal irradiation yet. In this paper stimulating effect of low dose ^{147}Pm internal radiation on DNA repair in spermiogenic stages is studied with alkaline elution technique.

2 MATERIALS AND METHODS

2.1 Experimental animals

Sexually mature male BALB/c strain mice, weight 24±1 g, were acclimatized to our laboratory for 7 d and every 5 were grouped in each polypropylene cage. Water and food were provided ad libitum throughout the period of experimentation.

* The Project Supported by National Natural Science Foundation of China
Manuscript received date: 1994-08-02

2.2 Sperm DNA labelling and ^{147}Pm injection

BALB/c mice are randomly divided into 5 experimental groups, and 1 parallel control group. There are 5 mice in each group. They are given intratesticularly (i.t.) injection of ^{3}H-TdR in dose of 167 kBq per testis for sperm DNA labelling. Spermatozoa are sampled after 36 d of labelling, it was just the time required for germ cells to mature. Animals with ^{3}H-TdR labeled are further treated using 5 different doses from 0.185 to 37 MBq/kg $^{147}Pm(NO_3)_3$ by intraperitoneal injection (i.p.). The period from i.p. injection of ^{147}Pm to sampling spermatozoa is fixed at 12 d as at this time the changes of alkaline eluted DNA from mice sperm treated with ^{147}Pm were more visible than other times.

2.3 Preparation of sperm cells

Both experimental and control mice are killed 36 d after ^{3}H-TdR labelling for preparation of sperm cells. The vas deferens from each animals were quickly removed and placed in 5 ml phosphate-buffered-saline(PBS) at pH 7.2 in the dark to prevent sperm DNA from light damage [5,6]. In 30 min, most of the sperm swam out of the vas into the PBS. Remaining sperm clumps were dispersed by gentle pipetting after the vas was removed from the PBS. This procedure resulted in very little trauma to the sperm and its mobility [7]. It was especially important that the number of sperm cells swimming into the 5 ml PBS should be larger than 5×10^{6} for assay.

2.4 Alkaline elution

Aliquot containing 5×10^{6} sperm cells was gently applied onto 2μm pore size polyvinyl chloride filter (25 mm, Millipore Corp., USA) which was placed inside a plastic filter holder and attached to a 50 ml glass syringe barrel, as shown in Figs.1 and 2.

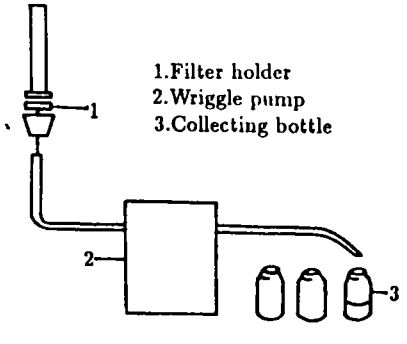

Fig.1 The alkaline elution equipment

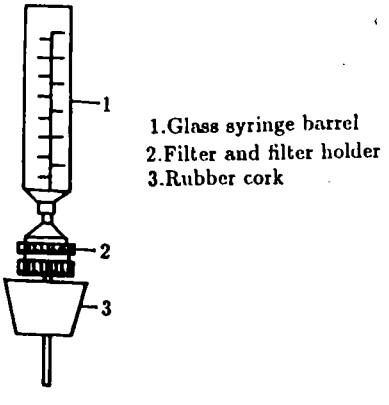

Fig.2 The filter equipment

Sperm cells were lysed by filling the syringe barrel with 5 ml lysis solution which contained 0.025 mol/l Na_2EDTA, 2% high purity sodium dodecyl sulfate (SDS), 1.0 mg/ml freshly dissolved cysteine and 0.5 mg/ml freshly dissolved proteinase K (Merck, Germany) adjusted to pH 9.6. All the lysis solution but not 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. After sperm cells have been lysed, the filter was eluted in the dark at a flow rate of 120 μl/min (adjusted by wriggle pump), using 30 ml of eluting buffer containing 2% tetrapropylammonium hydroxide (TPAH from Sigma Co. Ltd.) adjusted to pH 12.2, 0.02 mol/l acid EDTA and 0.1% high purity SDS. Fractions were collected for 4 h.

2.5 Measurement of DNA eluted

From each eluted DNA sample 5 ml was blotted 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 at 40°C and placed each in a scintillation vial. Of a scintillation mixture consisting of 100% toluene, 6.0 g/l PPO, and 0.6 g/l POPOP, 5 ml was added and the radioactivities of ^{3}H-labeled DNA were determined by liquid scintillation counting with the aid of a Beckman LS 6800.

3 EXPERIMENTAL RESULTS

3.1 The DNA on the filter and break down through filter

It was known that the damaged DNA fragments are shorter than the normal DNA chain, and the damaged sperm DNA after ^{147}Pm treatment easily eluted through the glass fiber filter by alkaline elution. The more the sperm DNA chain broken down, the less the DNA chain remained on the filter. The percentage of preservation of undamaged DNA on the filter could be calculated by the equation as follows: Filter preservation %=The DNA on the filter(cpm)/ [The DNA on the filter(cpm)+The DNA eluted through the filter (cpm)]×100%

3.2 Relation between ^{147}Pm dose and amount of DNA eluted

Table 1 shows the relation between the dose of ^{147}Pm given to mice by i.p. injection

Table 1
Amount of sperm DNA remained on the filter after alkaline elution from mice treated with different doses of ^{147}Pm 12 days ago

Doses /MBq·kg^{-1}	Control	0.185	0.74	3.7	11.1	37
Percentage	37.4±15.6	66.2±7.8*	36.6±3.7	25.5±9.3*	24.6±5.5*	13.3±0.6**

*: P <0.05, ** P <0.01, Percentage — % of sperm DNA preserved on the filter

and the amount of the preserved sperm DNA on the filter after elution. The dose of ^{147}Pm ranged from 0.185 to 37 MBq/kg. Results show the different effects of the different doses of ^{147}Pm given to the mice. It is a matter of interest that after small dose of internal irradiation with 0.185 Bq/kg ^{147}Pm, an increase of the sperm DNA remained on the polyvinyl chloride filter was observed which was considerably higher than the control group. While doses of ^{147}Pm were increased progressively, the decreases of sperm DNA on the filter were observed. There was a champed relationship between the amount of the sperm DNA eluted and the ^{147}Pm doses. Amount of eluted sperm DNA became increased with the progressive increases of ^{147}Pm doses.

It seems that low dose internal radiation only with ^{147}Pm 0.185 MBq/kg had a tendency to stimulate DNA repair in spermiogenic stages.

4 DISCUSSION

The most common DNA damage measured in irradiated cells is the strand breakage. The identification of radiation-induced DNA lesions in cells is even more difficult than DNA in aqueous solutions. One important reason is that in the cell DNA is present only at 1% level. Hence it must be isolated from the other components. Specific labelling, e.g. with tritium can solve some of the problems[8]. When DNA is subjected from ionizing radiation, the changes at the sugar-phosphate moiety can lead to strand breakage and base release. Alkaline elution is a technique usually used to detect DNA strand breaks. This technique provides a sensitive measure for DNA strand break and is applicable to a variety of problems concerning DNA damage, repair and its replication. According to Sega, the TPAH with stable pH transition zone extended from pH 11.6 to 12.8 was used as a suitable base for the sperm DNA elution[5].

It could be seen from our study that low level irradiation of ^{147}Pm had a tendency to stimulate DNA repair in spermiogenic stages as it was found that human lymphocytes exposed to low dose ionizing radiations of incorporated tritiated thymidine, were less susceptible to the induction of chromatid aberrations by subsequent high dose of X-ray. This adaptive response to further ionizing radiation, which occured after low dose exposures that were so low that they did not induce any aberrations themselves, but attributed to the induction of DNA repair that caused the restitution of X-ray induced breaks[9,10]. All these results suggest that stimulating effect of DNA repair exists in the cell, when they were treated with either internal or external low dose irradiation.

REFERENCES

1 Zhu Shoupeng. Radiotoxicology, Beijing: Atomic Energy Press, 1992; 214-220
2 Sagan L A, Cohan J J. Health Phys, 1990; 59(1):11—13
3 Zhu Shoupeng, Wang Yuanchang. J Radiat Res Radiat Proc, 1992; 10:12—19
4 Zhu Shoupeng, Wang Liuyi. Chin J Prevent Med, 1989; 23:166—170
5 Sega G A, Sluder A E, McCoy L S *et al.* Mutat Res, 1986; 159:55—61
6 Kohn K W, Erickson L C, Ewig R G *et al.* Biochem, 1976; 15:4629—4637
7 Sega G A, Generoso E E. Mutat Res, 1988; 197:93—99
8 von Somntag C. The chemical basis of radiation biology. London: Taylor and Francis, 1987; 271—279
9 Wolff S. In: Fielden E M, Fowler J F eds. Proceedings of the 8th International Congress of Radiation Research. Edinburgh:Taylor and Francis, 1987; 212
10 Planel H. Health Phys, 1987; 52:571—579